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JOURNAL OF ANATOMY

THE SPINAL CORD IN A CASE OF CONGENITAL ABSENCE OF THE RIGHT LIMB BELOW THE KNEE

By G. J. ROMANES, *Anatomy School, Cambridge*

The spinal cord described here was removed from a newborn female child showing several anomalies, including ectopia vesicae, a persistent neurenteric canal, two syringomyelocoeles confined to the lower lumbar and sacral regions of the spinal cord, variations in the segmentation of the lower lumbar and sacral vertebrae, and absence of the right lower limb from the knee joint region distally. The anomalies related to the ectopia vesicae have been described by Dr D. V. Davies (1942), to whom I am indebted for the details concerning the musculature of the right limb and for the spinal cord.

THE LIMB

The right limb ends abruptly in the region of the knee joint, and the femur as seen radiologically is essentially similar to that on the left side, both showing an epiphysial ossification for the lower end. In the stump there are two freely movable pieces of cartilage suggesting a patella and the upper end of the tibia. There is, however, no tibial epiphysial ossification to confirm this. This case is therefore almost identical with the congenital condition described by Walls in a woman of 75 years. None of the leg muscles are present, but the more proximal groups are essentially normal though smaller and less highly differentiated than on the left side. The main points are: (1) the quadriceps lacks the rectus femoris, (2) the gluteal muscles are very small and fused together. All the other muscles are present but show considerable degrees of fusion.

THE SPINAL CORD

The lumbo-sacral region of the spinal cord shows two syringomyelocoeles. The first, situated between the third and fourth lumbar segments, receives a large diverticulum from the central canal, and the second, in the sacral region, a small diverticulum from the central canal and a branch from the third right sacral nerve.

Owing to the irregular segmentation of the vertebrae in the lumbo-sacral region, the right fifth lumbar nerve is composed of two roots, a cephalic, consisting of a ventral root only, and a caudal, consisting of a small ventral root, a larger dorsal root, and a ganglion which is connected with the dorsal root of the fourth lumbar nerve. Similar fusions occur at other points in the lumbo-sacral enlargement and are most marked on the right side (Fig. 1).

In transverse section the right side of the spinal cord shows a reduction of the posterior white column. Opposite the roots forming the sacral plexus the posterior and anterior horns of the grey matter are also reduced (Fig. 2). There is no significant reduction in the anterior or lateral white columns.

From the fifth lumbar segment caudally the motor cell columns of the right anterior horn are considerably reduced compared with those on the left side (Fig. 2). The latter have been shown to be normal by comparison with the groupings found in the spinal cord of a human foetus of 14 weeks (Romanes, 1941), on the basis of which the following description is given.

Cephalic to the fifth lumbar segment the motor cell columns are the same in both right and left anterior horns of the grey matter, though those on the right contain fewer cells than those on the left. In the fifth lumbar and upper part of the first sacral segments the large motor cells of the right anterior horn are reduced to a few scattered in its most ventral part and representing the ventro-lateral group (columns 2 and 3). In the middle of the first sacral segment the central column (column 5) reappears and is continued into the second sacral segment where it ends. In the lower part of the second sacral segment a few cells appear in the position of the postero-lateral column (columns 6 and 7), but they are not numerous and are limited to a short extent of the cord. The ventro-medial cell column (column 1), which is absent from the fifth

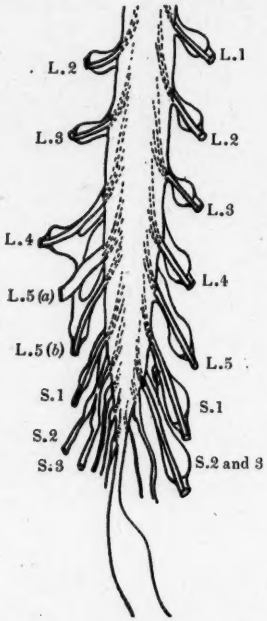

Fig. 1. A diagram of the ventral aspect of the spinal cord in the lumbo-sacral region.

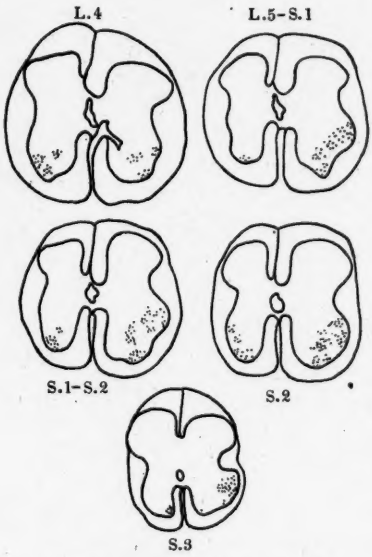

Fig. 2. A series of diagrams of transverse sections through the lumbo-sacral region of the spinal cord. ×12.

lumbar to the third sacral segments inclusive in the normal human spinal cord, reappears in the lower part of the third sacral segment and is larger in the right anterior horn than in the left, the latter being smaller than normal. This reduction of the ventro-medial cell column in the left anterior horn may be associated with a deficiency of the trunk mesoderm; this is evidenced by the lack of an umbilical artery on this side, whereas the right umbilical artery is intact.

No further cell columns could be found in the right anterior horn of the lumbo-sacral region of this spinal cord, and so, with the exception of the few cells mentioned above, the postero-lateral (columns 6 and 7) and the post-postero-lateral (column 8) cell columns are missing. Many authors (Bruce, 1901; Marinesco, 1904; de Neef, 1901; v. Gehuchten & Nelis, 1899; v. Gehuchten & de Buck, 1898) agree that these two columns

supply the leg and foot respectively, the former column, according to Marinesco, being split into two parts representing medial and lateral popliteal nerves. Thus the peripheral mesodermal loss is accurately represented in the motor cell columns of the spinal cord. Further confirmation of this has been found in the case of a 40-day-old rabbit where the right foot was missing from the level of the navicular bone distally, presumably having been bitten off by the mother just after birth. All the small muscles of the foot are absent, and the leg and thigh muscles are intact though some of the former have abnormal insertions. In the lumbo-sacral region of the spinal cord the post-postero-lateral motor cell column of the right ventral horn is completely lost though all the other cell columns are normal.

Reductions of the cell columns in the spinal cord have been shown in cases of congenital abnormalities similar to this in the adult (Edinger, 1882; Elders, 1910, 1914; Souques & Marinesco, 1897), in full-term foetal material (Curtis & Helmholz, 1911; Walls, 1940) and in cases of experimental embryonic, foetal or larval amputation (d'Abundo, 1914; Barron & Barcroft, 1938; Hamburger, 1934; May, 1930; Shorey, 1909). Results after adult amputations are far from clear, reduction of the anterior horn cells being found by some (Brissaud & Bauer, 1904; Campbell, 1896; Darkschewitsch, 1892; Dejerine & Mayor, 1878; Dickinson, 1868; Dickson, 1873; Dreschfeld, 1880; Friedlander & Krause, 1886; v. Gehuchten, 1898; v. Gehuchten & de Buck, 1898; Genzmer, 1876; Hayem, 1873; Hayem & Gilbert, 1884; Homén, 1888, 1890; Johnston & Lochart Clarke, 1868; Kahler & Pick, 1880; Lochart Clarke, 1868; Marie, 1892; Marinesco, 1892; Pellizzi, 1892; Prevost & David, 1874; Wille, 1895) and denied by others (de Buck, 1904; Dejerine & Mayor, 1878; Dejerine, M. & Mme, 1909; Lochart Clarke, 1866; Taft, 1920–1; Vanlair, 1891; Vulpian, 1868 *a*, *b*). The discrepancies in these results are almost certainly related to the difficulties found in attempting to produce chromatolysis in the anterior horn cells of the spinal cord of adult mammals by section or other damage to peripheral nerves (Romanes, 1941). It thus seems that adult mammalian material (Dejerine, M. & Mme, 1909) as compared with foetal or young material (Vulpian, 1868 *a*, *b*) is unsuitable for experimental researches into localization within the cell groups of the spinal cord.

While the above findings are valuable in determining central-peripheral relations of the spinal cord and limb muscles, they throw no light on the mechanism of development, only showing a constant correlated lack of mesoderm and neural tissue (see also Hepburn & Waterston, 1904; Pressy & Cobb, 1928; Yu-Ch'üan Tsang, 1939) without explaining the relation of these two parts to each other. However, experimental embryology has shown that removal of mesoderm brings about a loss of motor-neural tissue (Barron & Barcroft, 1938; Hamburger, 1934; May, 1930; Rogers, 1933; Shorey, 1909; Szepsenwol & Goldstein, 1938), though this is denied by Detwiler (1929) working on Urodeles. It has also shown that an increase in the mesodermal tissue causes hypertrophy of sensory and motor neural tissue (Detwiler, 1920; May, 1933). Conversely, removal of neural tissue has no primary effect on the developing mesoderm (Harrison, 1904; Hoadley, 1925; Murray, 1926; Wieman, 1926), and no description has been given of increase in the mesoderm as a result of experiments causing an increase in neural tissue in particular spinal segments (Detwiler, 1933; Severinghaus, 1930; Zacharias, 1938). The only suggestive evidence is that of Harrison (1924), but the researches of Wieman (1926) have shown that the mesodermal increase described is not due to the nervous system.

It seems likely therefore that the lack of mesoderm causes a decrease in the cells of the central nervous system rather than the reverse, especially since this foetus shows other evidence of a developmental insufficiency of mesoderm and, in cases so far described, developmental decrease of the mesoderm is always associated with neural reduction. On the other hand, developmental anomalies of the spinal cord have been described in foetuses showing an essentially normal arrangement of the peripheral mesoderm supplied by the abnormal spinal segments (Bruce, McDonald & Pirie, 1906, 1907; Holliday & Cormack, 1927; Kraus & Weil, 1926; Jenkel, 1907; Mefordiew, 1902; Schenk, 1934; Schneiderling, 1938; Solansky, 1930; Tretiakoff & Ramos, 1921; Weber, 1928). This means that in later stages of development the spinal cord is as dependent on the peripheral mesoderm for its completion as the early neural plate is dependent on the mesoderm for its formation (Holtfreter, 1933).

SUMMARY

The spinal cord of a newborn child whose right lower limb is missing from the knee joint distally is described. Marked reduction of the right side of the spinal cord in the lumbo-sacral region is noted. This affects the posterior column, and, opposite the roots of the sacral plexus, the posterior and anterior horns of the grey matter, the latter showing an almost total loss of the postero-lateral and post-postero-lateral motor cell columns which supply the leg and the foot. The loss of these cell columns of the anterior horn is considered to be the result and not the cause of the developmental insufficiency of the peripheral mesoderm.

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OBSERVATIONS ON THE LACERTILIAN SYMPATHETIC SYSTEM

By W. E. ADAMS, *Anatomy Department, University of Leeds*

Among the numerous references to the sympathetic nervous system in the Reptilia, those relating to the lacertilian sympathetic are surprisingly few. The earlier accounts of Fischer (1852), Hoffmann (1888) and Jaquet (1901), since they concern only the cervico-cranial sympathetic, have contributed little to an intelligent understanding of the organization of the sympathetic system as a whole. It has indeed only comparatively recently been shown (Hirt, 1921) that in the Lacertilia, as in mammals, there is a continuous paravertebral chain of ganglia extending from the thoracic inlet as far as the tail, and that these ganglia are connected with the spinal nerves by rami communicantes. Hirt postulated that between the limb plexuses these rami might prove to be mixed, i.e. to contain both preganglionic connector fibres proceeding from the spinal cord by way of the ventral root and postganglionic excitor fibres passing from the ganglia to a peripheral distribution with the spinal nerves. This hypothesis has since been substantially confirmed by von Reibnitz (1923) who showed, embryologically, that the preganglionic outflow actually takes place between the 9th and 24th spinal segments. Terni's description (1931) of the cervical sympathetic in the Lacertilia aligns itself with these recent accounts.

In a previous investigation (Adams, 1939) the nature of certain specialized cells situated in the adventitia of the carotid arch adjacent to the epithelial body III was discussed and the importance of determining their nervous connexions stressed, since Trinci's assumption (1912) that they were 'paraganglionic' implied a close relationship with the sympathetic nervous system, whereas it appeared on other grounds that they might be vaso-sensory cells comparable to those of the mammalian glomus caroticum. It soon became obvious, pursuing this problem, that our knowledge of the lacertilian sympathetic and (particularly) of its peripheral connexions was quite inadequate, and that a more thorough investigation of these was desirable. For this purpose, therefore, numerous specimens of *Lacerta viridis* were examined. The topography was largely studied by dissection, macroscopic and microscopic, the identification of the finest filaments being facilitated by the picric acid-borax carmine method proposed by Hirt. This was used in preference to the osmic acid method which Young (1931) found so useful in his investigation of the sympathetic of teleostean and selachian fishes, for the latter has not proved so effective in the Lacertilia. Serial sections of the head and neck were also prepared by vom Rath's technique (1895), but they did not produce the satisfactory results in *Lacerta* which Willard (1915) obtained in *Anolis*. Finally, the internal organization of the sympathetic system was investigated as far as possible by whole mounts of localized portions stained by osmic acid, and by Weigert preparations of sectioned material. I would like to record the valuable co-operation of Mr A. W. Dent of this Department in the various technical procedures which were attempted; I am grateful also to Mr S. R. Scarfe, of Middlesex Hospital Medical School, for the preparation of the photomicrographs.

OBSERVATIONS

In *Lacerta* the sympathetic trunk is present bilaterally as a continuous paravertebral chain of ganglia which extends from the thoracic inlet into the tail. The uppermost ganglion of the chain ('1st thoracic ganglion' of Hirt) is the largest and is situated in front of the brachial plexus at the level of the 6th spinal nerve (Fig. 1). Below this the ganglia become progressively smaller, and in the lower thoracic and abdominal regions are barely perceptible to the naked eye; they are segmentally arranged.

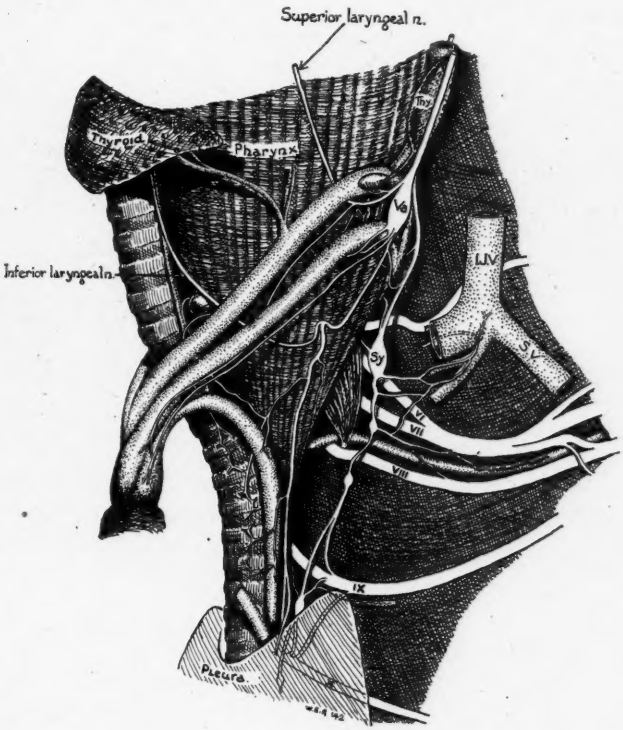
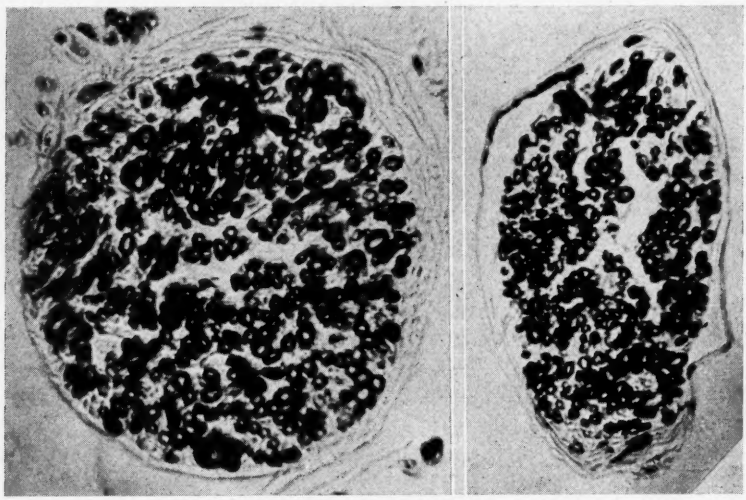


Fig. 1. Semi-diagrammatic view of the cervico-thoracic junctional region, showing the topographic relationships of the sympathetic trunk (*Sy*) to the brachial plexus, vagus nerve (*Va*) and pharynx. The vagus nerve and its principal branches are also depicted. *I.J.V.*=internal jugular vein; *S.V.*=subclavian vein.

Between the limb plexuses the ganglia are connected with the emerging spinal nerves by slender rami communicantes which in osmic preparations clearly show both medullated and non-medullated fibres; the former turn in towards the central end of the spinal nerve, while the latter run peripherally. This therefore also seems to support Hirt's contention that these rami contain both preganglionic and postganglionic fibres.

From the 11th spinal nerve caudalwards these rami invariably descend and become progressively longer, so that in the lower abdominal region the sympathetic trunk

takes up a somewhat more ventral position. In this region, also, numerous visceral branches are given off to the various parts of the alimentary tract, to the gonads, and to the kidneys (Spanner, 1928). Superiorly, above the 11th spinal nerve, the rami ascend, passing from the 8th, 9th and 10th nerves to join the sympathetic chain which just below this level is extremely fine. It seems therefore that the upper thoracic outflow runs almost exclusively cranially and the lower outflow caudally, while the slender trunk in between reflects the divergence of the two. The fact that Fischer was unable to trace the cervical sympathetic beyond its connexion with the nerves of the brachial plexus, suggests indeed that the section of the trunk immediately caudal to the plexus may occasionally be absent, although it was always so fine as to be easily overlooked unless particular care was taken.



A B

Fig. 2. Photomicrographs of transverse sections of cervical vagus (A) and sympathetic (B) trunks, to show the medullated fibres present in each. Weigert preparation.

The rami from the 9th and 10th nerves are both considerably larger than the main trunk at the same level (Fig. 3) and osmic preparations show that they contribute numerous medullated fibres to the sympathetic trunk, thus accounting for a significant feature of the lacertilian sympathetic, viz. that its cervical section is larger and more obvious than the remainder of the trunk. Also to be correlated with this is the fact that the uppermost ganglion of the trunk, situated in front of the 6th spinal nerve, is by far the largest.

While there can be no doubt that a great number of the medullated fibres derived from these two rami terminate in one or other of the upper three ganglia, it is equally certain, from osmic preparations, that a certain proportion of them continue uninterruptedly through these ganglia into the cervical trunk. Weigert preparations of the latter clearly show that it contains numerous medullated fibres, resembling in this respect sections of the vagus nerve at the same level (Fig. 2). While it cannot be

denied that certain of these fibres may be afferent in function it appears very probable from osmic preparations that others are preganglionic fibres which have traversed the upper thoracic sympathetic without synapse, and which presumably are then transmitted by the cervical trunk into the head.

Numerous postganglionic fibres proceed from the uppermost thoracic ganglia to various destinations (Fig. 3):

(*a*) One or two fine branches pass medially deep to the prevertebral muscles. These join to form a slender trunk ('deep cervical part' of Fischer) which ascends in front of the cervical spinal nerves and communicates with each of them by a fine ramus. Hirt has regarded this trunk as representing the homologue of the mammalian vertebral plexus although in *Lacerta* no vessel comparable to the vertebral artery of mammals accompanies it. It has not been possible to confirm Hirt's suggestion, which is supported by Terni, that this trunk affords a means of distribution of postganglionic fibres to the cervical nerves, nor could its connexion with the hypoglossal nerve, which he describes, be identified.

(*b*) Several filaments, passing laterally, unite to form a substantial cardiac branch which accompanies the superior vena cava on each side to the heart. This branch undoubtedly corresponds to that labelled by Hirt in his Fig. 5 as a 'branch to the carotid artery', since no branch was identified as passing to the carotid artery from any of these ganglia.

(*c*) Several small rami, which are certainly postganglionic in nature, join the upper trunks of the brachial plexus and also contribute to a vascular plexus around the subclavian artery (Fig. 3). Such a vascular distribution indeed appears to predominate in the thoraco-abdominal region of *Lacerta*.

Above the superior ganglion the sympathetic trunk continues upwards into the neck. As already mentioned, this is the most substantial part of the whole sympathetic and is comparable in size to the vagus with which it is closely related. It joins the internal carotid artery and lies with it at first lateral and then posterior to the pharynx. This portion of the trunk is non-ganglionated and has no communication whatever with any of the cervical spinal nerves. Furthermore, its connexions with the vagus nerve are not as substantial as is usually described although a fine communication between the two is often seen. Its branches are exceedingly few and extremely slender. One or two pass to the thymus glands and occasionally it was possible to identify an exceedingly fine branch passing to that region of the carotid arch which lodges the specialized 'glomus cells' (Fig. 1). The principal supply to this region, however, is undoubtedly derived from the superior laryngeal branch of the vagus, which accords with the view that the cells in question cannot be regarded as predominantly appendages of the sympathetic nervous system, and that they are therefore not paraganglionic in the sense which Trinci regarded them.

Just caudal to the hinder cranial nerves the cervical trunk, which suffers no diminution in size in its course throughout the neck, divides into two trunks:

(*a*) *A larger medial trunk* ('deep cranial part' of Fischer; 'cephalo-carotid ramus' of Terni) which conveys rostrally the majority of the medullated fibres which have been travelling up in the cervical trunk. In company with the internal carotid artery this trunk passes medially in front of the hinder cranial nerves to reach and join the ramus palatinus of the 7th cranial nerve at its commencement. In *Lacerta* therefore, and probably in other Saurians, the ramus palatinus is a composite nerve, containing

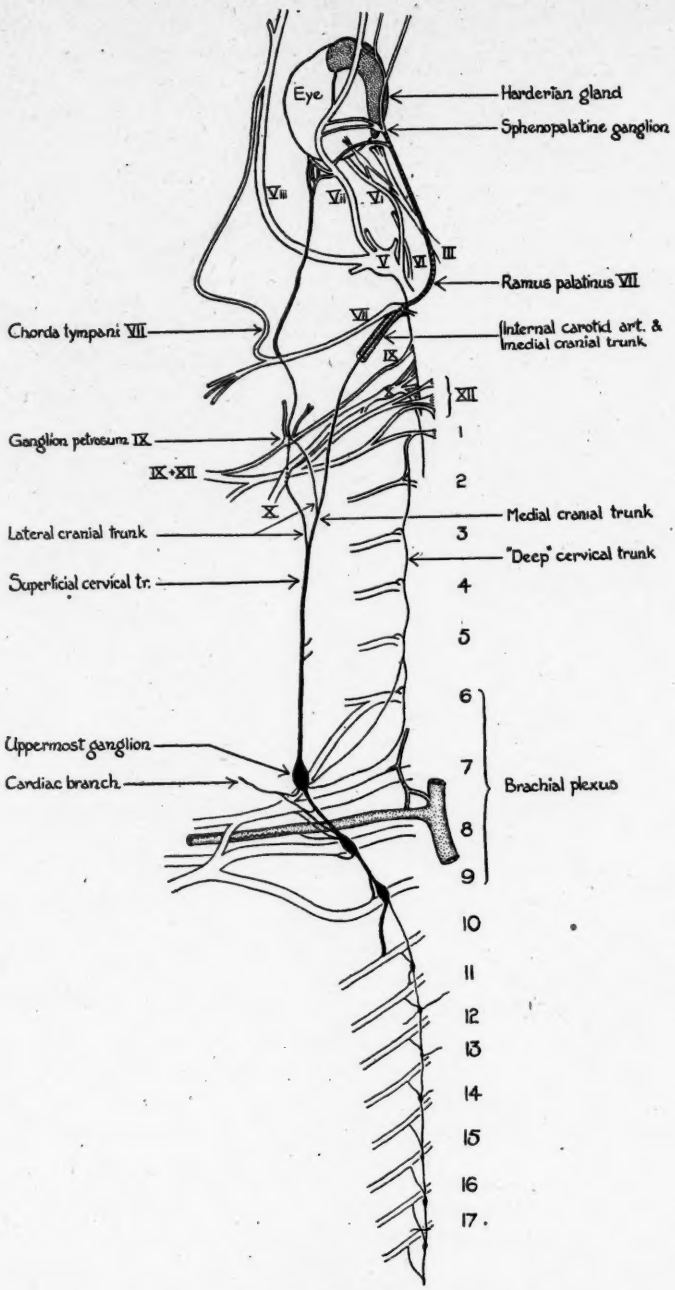


Fig. 3. A diagrammatic view from the ventral aspect of the cervico-cranial sympathetic to show its relationship and connexions with the spinal and cranial nerves. Roman numerals for cranial nerves, arabic for the spinal nerves.

numerous medullated and non-medullated fibres of sympathetic origin as well as fibres proper to the nerve itself and therefore either parasympathetic efferent or viscero-sensory fibres. As we have seen, the medullated sympathetic fibres are possibly, in part at least, preganglionic in nature, and the problem of their termination thus becomes one of considerable interest. Unfortunately, however, it has not been possible to trace them beyond the point at which they join the ramus palatinus. Nevertheless, the intimate relationship of the ganglion sphenopalatinum to the ramus palatinus of the seventh cranial nerve (Fig. 3) raises the possibility that they may terminate there and that this ganglion in the Lacertilia may therefore be composed both of sympathetic and of parasympathetic excitor cells.

(*b*) *A smaller lateral trunk* ('superficial cranial part' of Fischer) consisting principally of non-medullated fibres, the significance of which has not been elucidated. It may arise as one or two branches from the cervical trunk, which then ascend in front of the vagus and hypoglossal nerves to reach the ganglion ('petrosum') of the 9th cranial nerve. This trunk frequently passes through the ganglion or through the nerve adjacent to the ganglion, but there is no real evidence in favour of the view which has been advanced that this ganglion represents a sympathetic cell station, i.e. is equivalent to the superior cervical ganglion of mammals. Such medullated fibres as are present in the lateral trunk clearly traverse the glossopharyngeal nerve without interruption. Furthermore, it is not infrequently the case that the lateral trunk crosses the 9th nerve without joining it, the ganglion nevertheless still being present. The lateral trunk continues rostrally to join the 7th nerve (which it appears to traverse in a similar manner) and proceeds onwards, passing beneath the skin of the forehead region and then deeply behind the orbit to join the 2nd division of the trigeminal nerve. It has not been possible to determine the nature and destination of the constituent fibres of the lateral cranial trunk.

SUMMARY

The general topography of the lacertilian sympathetic is briefly discussed. It is confirmed that a continuous paravertebral chain of ganglia exists bilaterally and that between the limb plexuses these ganglia are connected with the spinal nerves (9–24) by rami communicantes which contain both preganglionic and postganglionic fibres. The majority of the former arise from the 9th and 10th spinal nerves and are distributed rostrally, thus contributing to the relatively greater size of the cervico-cranial sympathetic. It is suggested that certain of these preganglionic fibres proceed beyond the uppermost ganglion of the chain and ultimately reach the head. Their destination has not been determined but the possibility that they may terminate in the sphenopalatine ganglion is discussed.

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EUDIARTHRODIAL JOINTS IN FISHES

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INTRODUCTION

The work of Bernays (1878), Sémon (1899) and Lubosch (1910, 1938) has led to the classification of the several types of joint in a morphological series. From the simplest to the most complex forms these are: (1) synarthroses, in which the skeletal parts are attached to one another by cartilage, fibro-cartilage, fibro-tendinous or connective tissue (Fig. 3); (2) schizarthroses, in which a number of separate cavities appear in these tissues; (3) hemidiarthroses or periarthroses, in which there is a single joint cavity occupying the centre of the joint while the articulating elements are still united round the periphery; and (4) eudiarthroses, in which the articulating elements are separate, the joint cavity being limited peripherally by synovial tissues (Fig. 1). The authors quoted are also agreed that these morphological forms represent a true evolutionary series, suggesting that the typical Selachii, Dipnoi, Chondrostei and Holostei, which are said to possess only the first three of the above types, are primitive in this respect. On the other hand, those groups of animals to which the possession of eudiarthroses is said to be confined, the Raioidei, Teleostei and Tetrapoda, are considered to have acquired these structures as a new evolutionary advance which has been made independently in each group.

Lubosch (1910, 1938) considers that in urodele Amphibia the eudiarthroses found in the shoulder, elbow and hip, with their synovial structures and capsules, are new formations, and that it is doubtful whether those forms which do not now possess them, such as *Proteus*, ever did possess them. Schwarz (1935), on the other hand, from a detailed study of the wrist joint in several urodeles, has decided that the forms which have a well-developed joint cavity of hemidiarthrodial type, such as *Salamandra*, are the more primitive, while those, such as *Necturus* and *Amphiuma*, in which the cavity is small, are secondarily degenerate in this respect. Lubosch regarded this interpretation as doubtful, but in a recent study of the knee joint (Haines, 1942*a*) a similar conclusion to that of Schwarz was reached. This study provided evidence that in primitive tetrapods, including the ancestors of the urodeles, there was a fully developed eudiarthrodial joint with menisci and cruciate ligaments, but that these have been partially or wholly lost in modern forms. On the other hand, as already noted, it has been generally agreed that eudiarthroses are not found in the more primitive types of fishes, and are new developments in tetrapods.

Yet in the figures illustrating a study of the microscopic structure of the jaws of fishes (Haines, 1937) there are depicted, incidentally to the main theme, what are obviously fully developed eudiarthrodial joints, similar in every essential to those of tetrapods, and this in a series including the primitive types *Polypterus*, a direct descendant of the early palaeoniscids (Romer, 1933), and *Protopterus*, one of the surviving lungfish, as well as several Teleostei. It has needed only the addition of the two surviving holosteans, *Lepidosteus* and *Amia*, both fortunately available from the Zoological Society's collection, to complete a series indicating that the eudiarthrodial condition is primitive in the jaw joint for all bony fishes. These specimens will be described and

figured here, and the bearing of the conclusions reached on the phylogenetic development of joints may then be discussed.

OBSERVATIONS

Lepidosteus osseus. In the jaw joint (Figs. 1, 2) the quadrate (*Quad.*) is well ossified, and carries a convexity formed of a layer of calcified cartilage (*cal.ct.*) overlaid by a layer of hyaline cartilage (*hyl.ct.*). The cells of the calcified cartilage are hypertrophied,

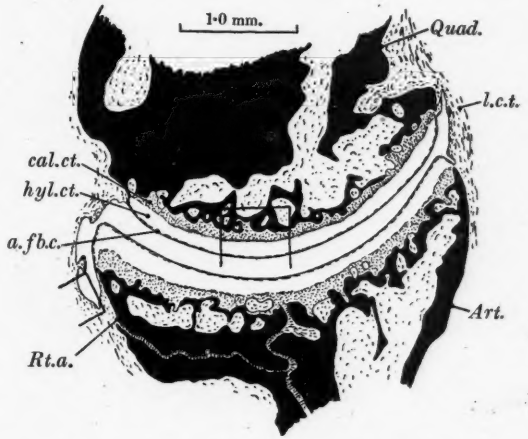

Fig. 1. The jaw joint of *Lepidosteus osseus*, to show a fully developed eudiarthrodial joint in a primitive type of bony fish.

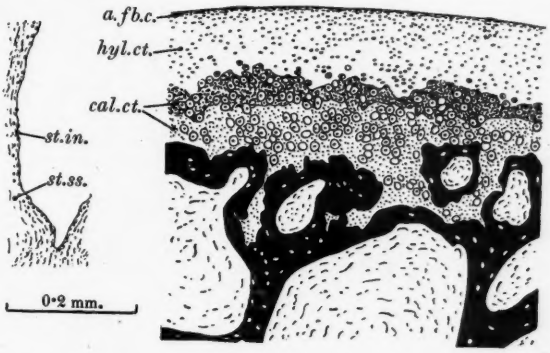

Fig. 2. Enlargements of the parts marked off in Fig. 1, showing a part of the synovial membrane and a part of the articular cartilage of the quadrate bone.

while those of the hyaline cartilage are smaller and rounded. Near the articular surface the cells are flattened and the matrix shows a thin acidophilic fibro-cartilaginous layer (*a.fb.c.*) underlying the surface itself. The mandibular articular surface is supported by two bones, the articular (*Art.*) and retro-articular (*Rt.a.*) which ossify in Meckel's

cartilage, whose structure resembles that of the quadrate cartilage. Peripherally the joint is surrounded by loose connective tissue (*l.c.t.*), and inside this is a fully differentiated synovial membrane, with the two layers of classical description (Lubosch, 1938), a stratum intimale (*st.in.*) and a stratum subsynoviale (*st.ss.*). The tissue is not sufficiently well preserved for more detailed description, but its general arrangement, and that of the whole joint, resemble closely those of a tetrapod (cp. Fig. 6), and indeed can hardly be distinguished without a close examination of the osteocytes.

The joint between the epi- and pharyngo-branchial elements of the third (first true) branchial arch (Fig. 3) is of the simplest possible construction, a relatively thin layer of fibro-cartilage (*fb.ct.*) uniting the cartilaginous epiphyses of the two bones to form a simple synarthrosis. Peripherally this layer passes gradually into the connective tissue of the perichondrium (*p.c.t.*).

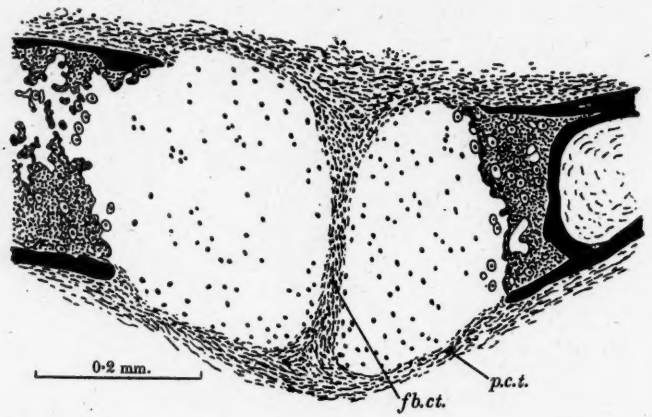

Fig. 3. The pharyngo-epibranchial joint of the third branchial arch of *Lepidosteus osseus*, to show a very simple type of joint co-existing with the complex type shown in Fig. 1.

In the fins the joints (figured by Lubosch, 1910, Fig. 34) are hemidiarthroses. The joint surfaces are not so smooth as in the jaw joint, but the cavity is well developed, though the bones are still joined at their periphery by a ring of cartilaginous and fibro-cartilaginous tissue.

Amia calva. This is a rather advanced holostean type, so that it is less likely to illustrate primitive conditions than *Lepidosteus*. The jaw joint is again eudiarthrodial, but the hyaline cartilage is covered with a thick layer of articular fibro-cartilage. The branchial joints, of much greater absolute size than in *Lepidosteus*, may show small joint cavities in the articular tissue (which is a mass of fibro-cartilage containing areas of hyaline cartilage), and the joints must therefore be regarded as poorly developed schizarthroses.

In the pectoral fin Lubosch (1910) has figured a hemidiarthrodial condition at the articulation of the proximal radial with the girdle and with the basal cartilage. In my specimen there is a well-defined joint cavity with occasional strands of connective tissue projecting into it from the articular surfaces. Peripherally there is

a synovial membrane with its two strata, so that the joint has reached a eudiarthrodial condition.

Joints of bony fishes. In the primitive fishes already described examples have been found of all the main types of joints met with in vertebrates, and this is also known to be the case in other bony fishes. The development of a wide range of varieties of joint must therefore be regarded as normal in these forms. In the dipnoan *Protopterus* (Haines, 1937) the jaw joint is a eudiarthrosis, though the quadrate and Meckel's cartilage remain entirely hyaline throughout life, for they are never calcified or replaced by endochondral bone or marrow. These peculiarities are, however, secondary, for, as Watson & Gill (1923) have shown, in the more primitive dipnoans of Devonian times there was a well-developed articular bone supporting the articular cartilage. Thus in these early forms the resemblance to the type of joint found in *Lepidosteus* must have been very close. In the joints of the fins of bony fishes Klaatsch (1896) and Lubosch (1910, 1938) have found hemidiarthroses, schizarthroses and synarthroses, the more highly developed condition being usually present in the larger joints, and the less highly developed in the smaller joints—particularly in the distal parts of the fins. The actual tissues that form the synarthroses differ in the three existing genera, being tendinous, fibro-cartilaginous or fibrous.

Again, in *Polypterus* the jaw joint (Haines, 1937) is a typical eudiarthrosis, with Meckel's cartilage ossified to form a well-developed articular bone, and with a hyaline articular cartilage covered with a thin layer of articular fibro-cartilage. In the early palaeoniscids *Elonicthys* (Watson, 1925) and *Nepatoptychius* (Watson, 1928) and the Devonian crossopterygian *Sauripterus* (Broom, 1913) the articular was well developed and carried a definite surface for the cartilage of the joint, so that here again there is every reason to believe that a eudiarthrosis was developed. In other parts of the body less highly developed joints have been described in the fins of *Polypterus* and *Calamoichthys* by Lubosch (1910), and my own sections from the branchial region of *Polypterus* show a structure similar to that in *Lepidosteus*.

Thus all these primitive types of bony fish agree in the possession in different parts of their bodies of joints of various types, but always with the most highly developed type in the jaw joint, and the least highly developed in the smaller joints of the branchial region and the distal parts of the fins.

In Teleostei (Lubosch, 1910, 1938) the various types of joint are well known as regards their general structure, and they are similar to those of more primitive fishes. However, a detailed study of a teleostean synovial joint by modern histological methods might well be of the greatest interest.

Elasmobranchs and sturgeons. In *Acipenser*, the only genus of sturgeons whose joints have been studied, Lubosch (1910) observed in one joint of the pectoral fin a small cleft, but otherwise he found only synarthroses. My own sections from the branchial region confirm these observations; on the other hand, the jaw joint is a schizarthrosis with several small joint cavities lying in a mass of connective tissue (Fig. 4). There can be little doubt, however, that such a structure in the jaw joint is secondarily derived from a eudiarthrodial condition, for, as Watson (1925) and others have shown, the modern Acipenseroidei have lost much of the bony structure of their palaeoniscid ancestors, and the jaws have been reduced to a weak protrusible apparatus.

The Elasmobranchii are more difficult to understand. In the fins of most Selachii, described fully by Bernays (1878) and Lubosch (1910, 1938) as showing what they took

to be relatively primitive conditions, there are found only synarthroses (all joints of *Carcharias*), schizarthroses and hemidiarthroses. In *Scyllium* and *Acanthias* the jaw joint is a hemidiarthrosis, while in *Zygaena* and in the holocephalian *Chimaera* (Fig. 5) it makes a closer approach to the eudiarthrodial state, with a synovial membrane

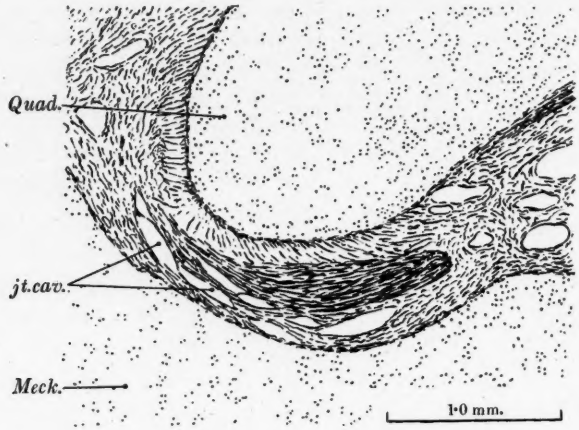

Fig. 4. Jaw joint of *Acipenser ruthenus*, to show a schizarthrosis associated with its predominantly cartilaginous structure, instead of the more usual eudiarthrosis.

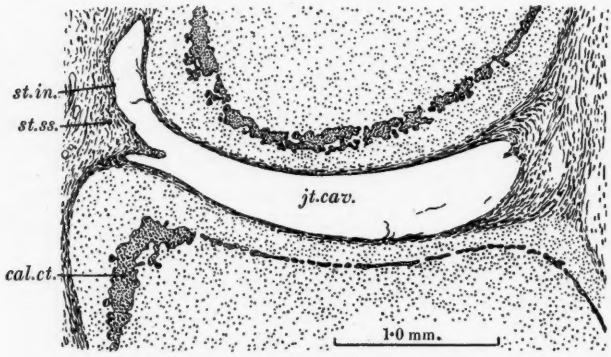

Fig. 5. Jaw joint of *Chimaera monstrosa*, to show an approach to the eudiarthrodial type of joint in an elasmobranch.

(*st.in.* and *st.ss.*) bounding the joint on one side. This partial development of a synovial covering is known also in urodeles (Lubosch, 1910), where it appears to be the result of a secondary simplification of a previously more highly developed type of joint. In the Raioidei, on the other hand, Lubosch (1910, 1938) has found well-developed eudiarthroses.

Whether the elasmobranchs as a whole ever had eudiarthrodial joints is uncertain.

They may have lost them, as have the modern sturgeons, for, though their ancestral history is unknown, there is good reason to believe that they are derived from forms with a more extensively ossified skeleton than they now have (Moy-Thomas, 1939).

Joints of tetrapods. In tetrapods the jaw joint has lost its position as the most highly developed joint in the body, though even in mammals it still maintains its identity as the malleolo-incudal joint. Apart from some of the more distal joints of the anurans, and most of the joints of the urodele amphibians, all the joints of the limbs are eudiarthrodial, and Fig. 6 illustrates the usual, and what is here believed to be the primitive, condition in tetrapods in general. The hyaline cartilage of the epiphysis is covered by a thin layer of articular fibro-cartilage (*a.fb.c.*), thickened in some parts of the joint to form a labrum (*lab.*). Wherever the joint cavity abuts on loose connective tissue there is a well-developed synovial membrane with the usual two layers (*st.in.* and *st.ss.*). The

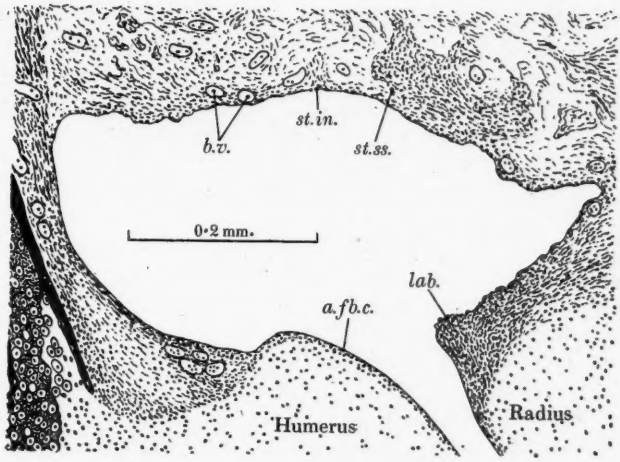

Fig. 6. Part of the elbow joint of *Emys orbicularis*, to illustrate what is here believed to be the primitive type of joint in the limbs of tetrapods, showing the synovial membrane with its stratum intimale and stratum subsynoviale, and the absence of any definite joint capsule.

subsynovial stratum is very vascular (*b.v.*), and blends without interruption with the connective tissue surrounding the joint, for there is no well-differentiated joint capsule. Over the dense fibro-cartilaginous tissue of the labrum only the stratum intimale is developed, for subsynovial tissue and blood vessels are absent. Other reptiles agree with *Emys* in the structure of their joints, including the primitive form *Sphenodon* (Haines, 1939, 1940; the animal figured by Lubosch, 1910, as '*Hatteria*' is, judging from the structure of its bone, a species of *Varanus*: see Haines, 1942*b*) and also numerous lizards, crocodiles and chelonians figured in the literature.

On the other hand, the urodele amphibians show much simpler types of joint; some of the larger and proximal joints are eudiarthrodial, while less complicated joints are found in the more distal parts of the limbs, particularly in the extremely neotenic forms such as *Proteus*, in which the phalanges may be joined by hyaline cartilage (Lubosch, 1910, 1938). But Schwarz (1935) has already given good reasons for con-

sidering such forms specialized even among the urodeles themselves, and the evolutionary changes that have led to the development of a tough, flexible type of animal from a more rigid and more bony type seem to have included with the reduction of dermal armour and of endochondral bone (Haines, 1942*b*) a simplification of the joints. Certainly the urodeles can no longer be expected to exemplify the more primitive types of joint structure.

THE SIGNIFICANCE OF THE JAW JOINT

Considering the evidence from Dipnoi, *Polypterus* and Holostei, there can be little doubt that one joint, the jaw joint, is always highly differentiated, and that this was also the case in the ancestors of these bony fishes. Further evidence on this problem would perhaps be obtained from a study of the living coelacanth of South Africa. The conclusion is reached that the jaw joint has led the way in joint evolution, being fully developed in Silurian times, when the bony fishes diverged into the various groups discussed (Holmgren & Stensiö, 1936), and that the other joints have followed the jaw joint in their progressive evolutionary development. Probably in any fish there is a potentiality for the development of a eudiarthrosis whenever a joint reaches a sufficient size and degree of movement, as for instance in the fin joint of *Amia*, but in the more primitive types the jaw joint alone satisfied these conditions, for the early semionotids, for instance, which include the ancestors of *Amia*, were all small fishes not more than 15 in. long (Brough, 1936).

This unexpected evolutionary precocity of the jaw joint suggests that eudiarthroses may be as old as the jaws and originally developed in association with them. The basic classification of vertebrates rests on the divergence of Agnatha, with, originally, a suctorial or sieving type of nutrition, and gnathostomes, with well-developed large jaws, particularly in early types, leading a predatory type of existence involving the capture of large prey by snapping jaws. There are no primitive gnathostomes now surviving, but the large ossified or calcified jaws of several of the early placoderms suggest the existence of a well-developed joint for Meckel's cartilage (Moy-Thomas, 1939). Hence it is possible that a fully developed jaw joint is a primitive inheritance of the Gnathostomata.

SUMMARY

1. Contrary to the usual opinion, eudiarthrodial joints with a single joint cavity and a fully developed synovial membrane similar to those of tetrapods are found in the more primitive types of bony fishes, including Dipnoi, *Polypterus* and Holostei, particularly in the jaw joint.

2. In tetrapods this type of joint, inherited from their piscine ancestors, has extended to include even the smaller joints of the limbs.

3. Eudiarthrodial joints were probably first developed in the common ancestors of the bony fishes in Silurian times, and may have been an essential part of the jaw mechanism that differentiated the Gnathostomata from the Agnatha.

My thanks are due to the Thomas Smythe Hughes Fund for a grant towards the purchase of material and to Profs. Appleton, Le Gros Clark and West for the gift of specimens.

KEY TO LETTERING

Bone is shown black, cartilage white and calcified cartilage stippled

a.fb.c.	articular fibro-cartilage	*lab.*	labrum
Art.	articular bone	*Meck.*	Meckel's cartilage
b.v.	blood vessel	*p.c.t.*	perichondral tissues
cal.ct.	calcified cartilage	*Quad.*	quadrate
fb.ct.	fibro-cartilage	*Rt.a.*	retro-articular bone
hyl.ct.	hyaline cartilage	*sd.c.t.*	strand of connective tissue
jt.cav.	joint cavity	*st.in.*	stratum intimale
l.c.t.	loose connective tissue	*st.ss.*	stratum subsynoviale

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THE PROBLEM OF NEURONAL REGENERATION IN THE CENTRAL NERVOUS SYSTEM

I. THE INFLUENCE OF SPINAL GANGLIA AND NERVE FRAGMENTS GRAFTED IN THE BRAIN

By W. E. LE GROS CLARK, *Department of Anatomy, University of Oxford*

In 1911, a report was published by Tello, from the University of Madrid, on a series of experiments in which fragments of peripheral nerve were implanted in the brain. The histological material appeared to demonstrate not only that neurones of the central nervous system have a capacity for regeneration similar to that of peripheral nerves, but that this capacity is stimulated to expression by neurotropic substances elaborated in degenerating nerves. Rabbits were used for the experiments, and small fragments from the peripheral stump of the sciatic nerve (cut 8–14 days previously) were placed within an incision in the cortex of the brain of other animals. These homografts were left in position for 12–40 days. Examination of the material showed newly regenerating fibres within the graft, and in some sections they could be seen penetrating the perineurium from the tissues of the host brain. In sections from a 14-day experiment, fibres of the white matter were seen converging from all sides towards the graft, though relatively few of these actually penetrated into the latter. Nevertheless, the orientated appearance of these fibres suggested that they had grown in response to some directive influence exercised by the grafted tissue. Indeed, Cajal (1928), in a reference to the experiment, remarks that it is 'as though they were attracted by an irresistible force'. Forty days after the insertion of a graft of predegenerated nerve, the latter was found to be undergoing resorption, and the regenerative phenomena which had been observed in the fibres of the host brain at an earlier date seemed now to be diminishing. Grafts of sciatic nerve not previously sectioned, and of the peripheral stump of the nerve into which regenerating axones had already penetrated from the proximal stump, did not lead to such striking results, though in the former case the regeneration of cerebral axones appeared to be in progress. Tello concludes from these observations that the best results are obtained when grafts are used in which Büngner's bands are well developed, and he infers, therefore, that the latter are the source of a specific neurotropic substance. In order finally to test this hypothesis, Tello made an extract, with normal saline, from the peripheral stump of a sciatic nerve which had been cut 8 days previously. Pieces of elder pith which had been soaked in the extract were then implanted in the brains of two rabbits. Of these, one died in 48 hr., and in this case the histological appearances seen in sections suggested an active growth of fibres from the white matter of the host brain into the pores of the implanted material. The other animal was killed after 14 days, but gave only negative results.

Tello's observations, and the conclusions which he draws from them, are clearly of the highest importance because of their theoretical implications, and also because of the practical possibilities which they suggest. It seems remarkable, therefore, that they have not led to further and more extended investigations by other workers in

the same field. In the present report, an account is given of work undertaken in order to determine the limits of regenerative processes which may occur in the brain in experiments similar to those employed by Tello.

In addition to the implantation of portions of predegenerated sciatic nerve, in some experiments spinal ganglia were grafted in the brain alongside nerve fragments. This was done (1) to determine how far the regenerative capacity of the ganglion cells is affected by the environment of the host brain, (2) to afford an opportunity for comparing the regenerative capacity of the ganglion cells with that of the cerebral neurones of the host, and (3) to see whether the grafted piece of sciatic nerve exerts any directive influence on the axonal outgrowths of the neighbouring ganglion cells. Incidentally, also, it has been claimed that the proliferating satellite cells, which form a conspicuous feature of grafted ganglia, are particularly rich in neurotropic substances. They might therefore be expected to exert a strong influence on the fibres of the host brain.

MATERIAL AND METHODS

Rabbits approximately 3 months old were used for the experiments. Portions of nerve for implantation were taken from the peripheral stump of a sciatic nerve cut 10–14 days previously. At the preliminary operation, the proximal stump was turned up so as to delay the arrival of regenerating fibres across to the distal stump. For the grafting operation, small fasciculi were removed from the peripheral stump at a distance of about 2 cm. from the level of the section. The host animal was prepared, before removing the graft from the donor, by a limited craniotomy to expose the site of implantation. In some cases the nerve graft was placed longitudinally within an incision made in the dorsal aspect of the parietal region of the cerebral hemisphere. In other experiments, the graft was introduced through a trephine hole near the mid-line, and with the help of fine curved forceps drawn across the mid-line below the surface of the brain towards a corresponding trephine hole on the opposite side. The nerve fragment thus came to occupy a position immediately dorsal to the corpus callosum, extending transversely from one hemisphere to the other across the longitudinal fissure of the brain. It was thought that such a location might provide more convincing evidence for the true regeneration of fibres if the latter were found to extend across the mid-line in this position. In one experiment, the graft consisted of the proliferating tissue ('Schwannoma') at the free end of the distal stump of a sciatic nerve which had been cut 7 weeks previously.

For the implantation of spinal ganglia, the latter were removed from the thoracic region in the donor animal, and placed in position in the cortex of the host close to one end of a grafted nerve fragment.

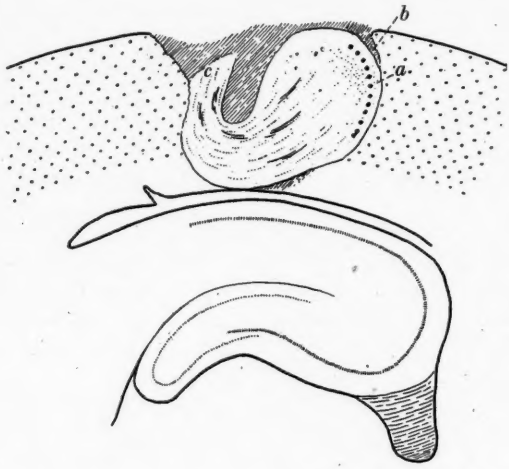
The host animals were killed 13–20 days after the implantation. In all experiments the rabbits were anaesthetized by intravenous nembutal, and the operations were carried out with strict aseptic precautions.

In each experiment, a block containing the implantation site was removed from the brain and serial sections were cut at a thickness of 10 μ. In some cases the blocks were treated by a pyridine-silver technique, and in others the sections were stained by Bodian's protargol method. The latter was found to be less suitable for the present purpose since it is not sufficiently selective to permit of the ready identification of solitary regenerating nerve fibres unless the latter are cut along their length.

OBSERVATIONS

Exp. 1 (R. 203). Operated 21 May 1941, killed 11 June 1941. A small fasciculus of predegenerated sciatic nerve from another animal, sectioned 11 days previously, 10 May, was implanted longitudinally beneath the surface of the cortex of the left hemisphere. From the same donor a spinal ganglion from the dorsal region of the cord was removed and also implanted in the cortex, close to one end of the nerve graft.

On exposure of the brain after death, the sciatic nerve graft was found to have been extruded from the brain and to be lying free on the surface of the cortex. Serial sections were prepared through the site of implantation and treated by the pyridine-silver method. The sections show that the grafted ganglion is firmly embedded in the cortex and has become vascularized from adjacent cortical vessels as well as by pial vessels extending in from the surface at the site of implantation. The position of the



Text-fig. 1. Diagram showing the position of the grafted ganglion in Exp. 1.

graft is shown diagrammatically in Text-fig. 1. The ganglion is separated from the surface of the cortex by a thin layer of vascular granulation tissue, and on its deep aspect it extends into the white matter practically to the roof of the lateral ventricle. Most of the ganglion cells have undergone complete degeneration and have been replaced by closely packed masses of small, rounded, and polygonal cells which have evidently resulted from the proliferation of the satellite cells (amphicytes). The appearance of these satellite cells in a normal spinal ganglion is shown in Pl. 1, fig. 2. In this methylene-blue preparation, a normal ganglion cell is seen enclosed within its mesothelial capsule (which evidently corresponds with the endoneural sheath of the peripheral nerve fibre). Within the capsule, and closely applied to its inner surface, are a number of small cells with round nuclei. These are the satellite cells which, on atrophy of the ganglion cell, proliferate and take its place—recalling, therefore, the behaviour of Schwann cells in the peripheral stump of a cut nerve.

Where the remains of the ganglion lie in contact with cortical tissue (at *a* in Text-fig. 1) the cell masses are not separated from the latter by any definite capsule of fibrous tissue—indeed the cortex itself appears to have been infiltrated to some extent by the proliferating cells, which thus form a zone intervening between the persisting ganglion cells and the relatively normal cortex of the host (Pl. 1, fig. 3). This zone is well vascularized by dilated capillary vessels and is also crossed by an occasional arteriole from the adjacent cortex (Pl. 3, fig. 27). On one side of the ganglion the remains of its central rootlets are seen. Emerging from the grafted ganglion they bend rather sharply towards the surface of the brain (*c* in Text-fig. 1). Their cut ends are separated from the surface by granulation tissue.

The persisting ganglion cells are found in a narrow row close to the periphery of the ganglion. Altogether, 123 surviving cells were counted in the serial sections. They show considerable variation in size, but many of them retain their normal characters, being rounded in shape, unipolar, with a well-defined nucleus (which, however, is frequently excentric in position) and a fibrillary reticulum in the cytoplasm. Others show the abnormal configurations which have been described by several authors (e.g. Nageotte, 1907; Marinesco, 1907; Agosti, 1911; and Cajal, 1928). The cell body may be elongated and constricted in the centre ('en fausse bipartition', Pl. 1, fig. 8), it may be irregularly lobulated, and in some cases it has become multipolar, sending out coarse 'paraphytes' which often end in large rounded bulbs or in complicated skeins (Pl. 1, fig. 6). Before proceeding further with a description of the new outgrowths, however, it is convenient to refer to the residual nodules of Nageotte. It has been noted that most of the ganglion cells have undergone disintegration and have disappeared. In some instances, the remains of the cytoplasm of an autolysed cell are to be seen, surrounded by numerous satellite cells which have proliferated within the mesothelial capsule of the cell. Where the ganglion cell has completely disappeared, its place is frequently occupied by a circumscribed nest of satellite cells—these clusters are the residual nodules (Pl. 2, fig. 14). Many of these nodules are destitute of nerve fibres. Others, on the contrary, are filled with a close-meshed network of fine fibres (Pl. 2, fig. 17). The latter are produced by the ramification within the nodule of fibre processes derived from neighbouring ganglion cells which persist. The processes either come directly from the cell body in those instances where the ganglion cell has become multipolar, or they may arise from the proximal part of the axone of the cell. In any case, it appears (as Nageotte, Cajal and others have recorded) that many of the residual nodules have become 'neurotized' by new fibre outgrowths, and it has been frequently suggested that this is the result of some chemotropic influence of the proliferated satellite cells which 'attracts' such outgrowths. The sprouts from the ganglion cells or from the proximal part of their axones may have other destinations. Some spin a complicated plexus within the capsule of the cell from which they arise, or of other, adjacent cells, forming pericellular networks (Pl. 2, figs. 10, 18). Others enmesh the glomerular origin of the axones, forming the well-known periglomerular plexuses (Pl. 1, fig. 6). Still others branch out into and become lost within the surrounding masses of cells, forming plexiform leashes which frequently end in bulbous swellings or terminate in beaded fibres (Pl. 1, fig. 4). It is well established that such formations as pericellular nets or periglomerular plexuses are to be found occasionally in apparently normal ganglia, but they are rare in the latter and are commonly regarded as abnormal structures. Cajal (1928) has pointed out that they are frequent, however, in ganglia

which have been damaged by disease or injury. Their presence in large numbers, and in an exaggerated form, in grafted ganglia is the result of the new outgrowth of processes from the surviving cells.

Still more interesting evidence of the new growth of nerve fibres from the ganglion cells is provided by those which penetrate out into the zone of cellular infiltration which separates the cells from the intact cortical tissue of the host, and by those which extend down into the endoneural sheaths of the rootlets of the ganglion. As regards the former, here and there such fibres are seen extending towards the cortex in the region marked *a* in Text-fig. 1. Most of them turn to run parallel to the surface of the grafted ganglion and soon end. Others, however, extend out farther towards the cortical tissue of the host, but none actually reach it. These fibres either end in rather large globular bulbs (such as the examples shown in Pl. 2, fig. 16), or they may take a recurrent course back towards the ganglion cells. At one point near the surface of the brain (marked *b* in Text-fig. 1) a stout blood vessel extends from the cortex across the zone of cellular infiltration towards the grafted ganglion (Pl. 3, fig. 27), and here a process of one of the ganglion cells extends along the wall of the vessel towards the adjacent cortex but, again, does not reach it.

The rootlets of the implanted ganglion consist mostly of endoneural sheaths devoid of nerve fibres but containing large numbers of vacuolated macrophages as well as strands of Schwann cells. Here and there, however, are well-defined nerve fibres, disposed either singly or in small compact fasciculi. Only a few of the fibres reach the free end of the rootlets; the majority soon end in irregular swellings at varying distances from the ganglion cells. There is good evidence that most, if not all, of these fibres are partly or wholly new outgrowths. Many of them, for example, show collateral branches and terminal expansions; they may assume a spiral or recurrent direction in part of their course; and, on occasion, they may be found within an old endoneural sheath alongside the debris of a degenerated axone. Again, a number of nerve fibres may be seen enclosed within a single sheath, a disposition which is commonly found in the peripheral stump of a nerve during the process of regeneration. An example of this is seen in Pl. 1, fig. 7, which shows such a fasciculus cut transversely. It is situated about half-way down the course of the rootlets, and it consists here of 25–30 fibres disposed in a circle closely applied to the inner wall of the endoneural sheath. Here and there (as seen in Pl. 1, fig. 7), one of the fibres gives off a collateral which pursues a circumferential course within the sheath. It is of interest to note that, when this occurs, the collateral usually (though not invariably) lies within the circle of nerve fibres, suggesting that the latter are here closely adherent to the inner wall of the sheath, and but loosely related to the cytoplasm of the macrophage elements which are contained within it. However, such a relationship evidently cannot be regarded as essential, for, if such a fasciculus is traced throughout its course in the serial sections, it is found that the constituent fibres are not consistently disposed in a ring. At intervals they are grouped rather in the centre of the sheath, in a fairly compact bundle, and not infrequently they intertwine with each other and tend to run spirally. The fasciculus shown in Pl. 1, fig. 7, was traced proximally to the immediate vicinity of the surviving ganglion cells. Here it consists of about ten fibres only. The ultimate origin of the fibres, however, could not be determined, for the fasciculus at its commencement becomes lost in a plexus of other fibres. Its disposition near its origin suggests that it is derived either from the multiple sprouting of a single axone or at

least from axones of cells which are closely grouped together. Traced distally, some of the fibres are seen to dichotomize so that the fibres within the single sheath increase in number. Others may give off short collaterals which wind spirally and end in bulbous swellings. Finally, towards the free end of the rootlets the fibres end in a tangle (Pl. 1, fig. 9), many of them presenting rather large terminal expansions or circling round the inside of the sheath in an approximately transverse plane ('apparatus of Perroncito'). It may be noted that an occasional single new fibre is to be seen running across the main course of the nerve sheaths and not itself obviously related to an endoneural sheath. Such fibres become lost in the connective tissue of the endoneurium.

The reaction of the neurones of the host tissue now requires consideration. It may be said at once that no conclusive evidence has been obtained of any but a degenerative reaction. The nerve cells and fibres of the cortex in the immediate proximity of the graft are intensely and clearly impregnated, and many of the fibres show well-recognizable traumatic reactions such as rounded and fusiform darkly staining bulbs, ring formations, beading, uneven thickening and other irregularities. In some sections the fibres of the white matter at the margin of the graft give the appearance of regenerative orientation in rather compact fasciculi directed towards the graft. But this appearance is certainly illusory. It is evidently the result of displacement and compression of pre-existing fibres by the inflammatory infiltration of the traumatized white matter. The atrophy of some fibre systems in the white matter, consequent on the destruction of cells of origin in the injured area of the cortex, further serves to bring into prominence the relatively isolated bundles of fibres which remain and which often stand out even more conspicuously because of their intense impregnation. It is necessary, therefore, to avoid mistaking such persistent fibre systems for the orientated outgrowths of new fibres. None of these fibres enters the grafted tissue, nor do they individually present the usual histological picture of regeneration. The part of the graft containing the rootlets is in many places separated from the host tissue by a thin but fairly compact fibrous capsule, and it may be supposed, therefore, that this acts as a barrier to the penetration of possible regenerating fibres from the cortex or the white matter. However, it is otherwise with the part of the graft which contains the ganglion cells and their remnants. In many sections this area is separated from the host cortex by a very thin zone of relatively loose tissue which is largely composed of small round cells. As already described, this tissue is evidently accessible to newly outgrown fibres of the surviving ganglion cells; nevertheless, the fibres in the host cortex show no tendency to penetrate it towards the grafted tissue. It is of particular interest to call attention again to the well-defined blood vessel which crosses from the host cortex towards the graft near the surface (at *b* in Text-fig. 1). It is well known that regenerating fibres have a propensity for extending along the walls of blood vessels, and, indeed, it has been noted above that some sprouting fibres from the grafted ganglion cells actually do so in this case. Yet, in spite of this obvious pathway, no nerve fibres from the cortex show any inclination to follow the same path in the reverse direction. Here and there, occasional cortical fibres are indeed seen in the cellular infiltration surrounding the graft (in the region of *b* in Text-fig. 1). Such a fibre is shown in Pl. 1, fig. 5, and in this case it is seen to extend for some distance towards the graft and then to pursue a recurrent course. It is possible that this represents an abortive attempt at regeneration. However, there is an alternative interpretation which a study of the sections makes more probable. This is, that it is

really nothing more than a pre-existing fibre in a zone of the cortex which has been invaded by a cellular infiltration from the grafted tissue, and that it has been distorted and displaced by this infiltration. It is an interesting fact that a close scrutiny of each one of the serial sections fails to show any place where the outgrowths from the grafted ganglion cells intermingle or come into contact with the fibres of the host cortex or white matter. Yet, so far as can be determined by histological appearance, there are regions where no obvious physical barrier separates the two systems. It may further be stated quite definitely that every nerve fibre found in the rootlets of the grafted tissue is a derivative of the surviving ganglion cells. Even where the cut ends of the rootlets reach close to the surface of the host brain, there is no sign of regenerating fibres from the cortex extending down into the empty sheaths.

The histological observations on this experimental material have been reported in some detail in order to emphasize that the grafted tissue clearly provides the opportunity for the growth and extension of regenerating fibres. This applies to the cellular tissue in the region of the ganglion cells as well as to the endoneural sheaths and Schwann cells of the degenerated rootlets. Thus, if it is the case that such regeneration is initiated by neurotropic substances elaborated by satellite cells or Schwann cells, or even if these elements simply provide the necessary pathways for the growth of nerve fibres on Dustin's principle of odogenesis, then, assuming that the cerebral neurones are endowed with regenerative potentialities similar to those of peripheral nerves, they might be expected to show some definite evidence of regeneration. As we have seen, however, the only definite evidence of regeneration in this experiment is provided by the surviving ganglion cells in the graft.

Exp. 2 (R. 200). Operation 21 May 1941, killed 4 June 1941. A small piece of pre-degenerated sciatic nerve from another animal, sectioned 11 days previously on 10 May, was implanted longitudinally beneath the surface of the cortex of the left hemisphere. From the same donor a spinal ganglion from the dorsal region of the cord was removed and also implanted in the cortex close to one end of the nerve graft.

Serial sections, treated by the pyridine-silver method, show the grafted nerve fragment to be firmly embedded in the host cortex, extending down to the roof of the lateral ventricle. The several fasciculi of the graft are separated from each other by layers of rather dense fibrous tissue. On the other hand, where the grafted tissue lies in apposition to the cortex and white matter of the host brain, it is covered by a thin and often indistinct layer of loose fibrous tissue. The endoneural sheaths are filled with numerous macrophages among which can be seen occasional axonal debris. In most parts of the graft only a few scattered Schwann elements seem to have survived, but in one region they persist in the form of vigorous-looking cytoplasmic bands. A systematic study of the sections fails to show any regenerating fibres in the grafted nerve, with the possible exception of one point near the surface where the appearance under the microscope suggests the presence of a few very fine fibres which may have extended in from the tissues covering the surface of the brain.

In close apposition to the grafted nerve fragment, the remains of the grafted ganglion are evident. No ganglion cells have survived in this experiment, though the necrotic remains of some of these can be recognized. Otherwise, their position is indicated by characteristic residual nodules, and by closely packed masses of small rounded cells. No nerve fibres are present in any part of this tissue, nor are any to be found in the degenerated rootlets of the ganglion.

The reaction of the tissue of the host brain is the same as that of the previous experiment. The cortical cells and fibres and the fibres of the white matter have been excellently impregnated, and adjacent to the implantation site the usual traumatic changes are to be noted. Nowhere, however, is there any evidence of attempted regeneration.

Exp. 3 (R. 201). The details of this experiment are similar to those of Exp. 2. However, the serial sections show that the implantation of the nerve fragment and ganglion had not been successful, for no trace of them could be found. There is no evidence of a regenerative process in the nerve fibres of the host brain that had been injured at the operation site.

Exp. 4 (R. 193). Operation 5 May 1941, killed 19 May 1941. A small fragment of predegenerated sciatic nerve was implanted longitudinally beneath the cortex of the left cerebral hemisphere. The graft was taken from another animal in which the sciatic nerve had been cut 14 days previously on 21 April.

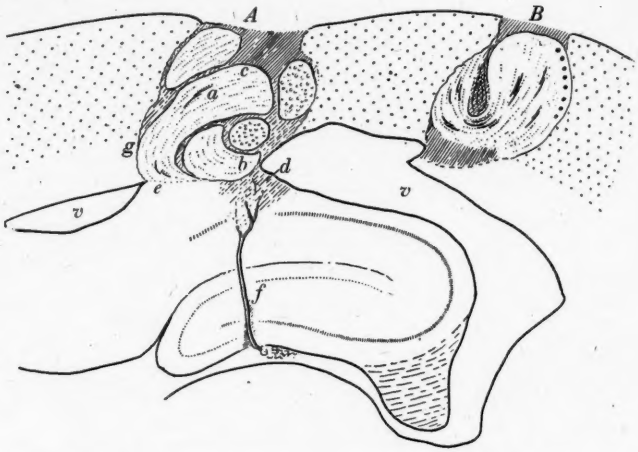
Serial sections of the implantation site were prepared by the protargol method. They show a successful grafting of the nerve fragment; the fasciculi of the latter extending from the surface of the cortex and through the subjacent white matter as far as the roof of the lateral ventricle. There has been considerable cellular infiltration of the host tissue all round the graft. The endoneural tubes of the grafted fragment are filled with macrophages and axonal debris. There is but little evidence of the formation of typical Büngner's bands, but here and there elongated Schwann cells are to be seen, particularly in the more superficially placed fasciculi. Nowhere in the graft is there any sign of the entrance of regenerating fibres. The nerve fibres of the host brain have been fairly sharply impregnated, and where they have been involved in the lesion at the implantation site they show many of the usual traumatic reactions, particularly in the formation of irregular beading and globular retraction bulbs. On the other hand, there is no histological evidence that any of these fibres have undergone even abortive regenerative processes.

Exp. 5 (R. 184). Operation 3 May 1941, killed 17 May 1941. A small fragment of predegenerated sciatic nerve was implanted under the cortex, extending across the mid-line from one hemisphere to the other in the region of the corpus callosum. The graft was taken from another animal in which the sciatic nerve had been cut 12 days previously, on 21 April.

Serial sections of the implantation site were prepared by the pyridine-silver technique. They show that the graft has been retained in position, and is firmly encapsulated by a layer of dense fibrous tissue. The grafted tissue is composed mainly of a honeycomb meshwork of endoneural sheaths, with little trace of surviving Schwann cells or axonal debris. Macrophage elements are also far less abundant than in the successful grafts of other experiments. There is no evidence of regenerating fibres in the graft. The impregnation of the cells and fibres of the host brain has not in this case been uniformly successful, and in the neighbourhood of the lesion the staining is rather diffuse. However, a study of the serial sections leads to the conclusion that there has been no outgrowth of new fibres in the brain tissue of the host, nor is there histological evidence of any regenerative processes.

Exp. 6 (R. 199). Operation 20 May 1941, killed 9 June 1941. A small fasciculus of predegenerated sciatic nerve was implanted longitudinally beneath the surface of the cortex of the left cerebral hemisphere. The graft was taken from another animal in which the sciatic nerve had been sectioned 10 days previously, on 10 May.

Serial sections, treated by the pyridine-silver method, show the implanted tissue firmly embedded in the cortex. It is encapsulated by a relatively thick layer of dense fibrous tissue which separates it almost everywhere from the tissues of the host. At one point, however, where one end of the grafted fasciculus lies in close relation to the cavity of the lateral ventricle, it appears to grade insensibly into granulation tissue which has invaded the subcortical white matter of the host brain. This granulation tissue contains many persistent fibres of the white matter which are sharply and clearly impregnated. However, neither here nor elsewhere is there any evidence of regeneration by the axones of the host. The grafted tissue consists of persistent endoneural sheaths containing axonal debris and many macrophages. It appears that only a few Schwann elements have survived here and there; indeed, although there is no actual necrosis, the tissues of the graft appear relatively inert. No regenerating nerve fibres are anywhere present in the graft.



Text-fig. 2. Diagram showing the position of the grafted nerve fragment (*A*) and the grafted ganglion (*B*) in Exp. 7. *v*, lateral ventricle.

Exp. 7 (R. 202). Operation 21 May 1941, killed 11 June 1941. A small piece of predegenerated sciatic nerve from another animal, sectioned 11 days previously on 10 May, was implanted longitudinally beneath the surface of the cortex of the left hemisphere. From the same donor a spinal ganglion from the dorsal region of the cord was removed and also implanted in the cortex, close to one end of the nerve graft.

Serial sections, prepared from the brain in the region of the implantation and treated by the pyridine-silver method, show that the grafts of both the sciatic nerve fragment and of the spinal ganglion have survived. They are firmly embedded in the cortex and are well vascularized by extensions from the cortical vessels of the host. The minimum distance between the adjacent margins of the two grafts is approximately 1·5 mm.

As may be seen in the diagram in Text-fig. 2, and in Pl. 1, fig. 1, the nerve graft extends through the subcortical white matter and the underlying part of the cavity of the lateral ventricle to reach the dorsal surface of the hippocampus, and has become adherent to the latter. The fasciculi of the nerve fragment have become somewhat

contorted so that in a single section four or five bundles may be seen cut across in different planes. Between the adjacent fasciculi the perineurium has become considerably thickened and rather dense. On the other hand, there has been very little organization of a capsular membrane over the fasciculi where the latter lie in contact with the cortex or with the white matter of the host brain. Indeed, at one point where the cut ends of the nerve sheaths lie in contact with and adherent to the hippocampus (at *e* in Text-fig. 2) there appears histologically to be no definite intervening layer or fibrous membrane separating the grafted from the host tissue; the endoneural sheaths with their Schwann bands here apparently lie in immediate contact with the fibre layer of the alveus. The tissues of the grafted nerve have an active and healthy appearance. They show an abundance of empty sheaths filled with macrophages and occasional remnants of degenerating axones, and in many places elongated protoplasmic strands of Schwann cells are arranged in fairly regular alignment (Pl. 3, fig. 28). Of outstanding interest for the present study, however, is the observation that the graft contains some newly regenerated nerve fibres which are quite evidently extending in a vigorous manner along the course of the endoneural sheaths and in intimate relation to the Schwann elements. These fibres are stout and intensely stained (Pl. 2, fig. 12). They usually run singly, but in one instance they form a compact bundle of about ten fibres enclosed in a common endoneural sheath. The regenerated fibres occur in two sets. Of these, one (consisting of 6–10 fibres) is found in a portion of the graft which curves down from the surface of the cortex to reach the surface of the hippocampus (*a* in Text-fig. 2). The fibres in this fasciculus can be traced section by section. They clearly follow a downward direction for, arching down towards the lateral ventricle, they thin out and end at different levels, sometimes in terminal expansions. When traced upwards, the fibres can be followed to the surface of the brain, where they enter the perineurium of the graft. They then disappear from view, for the impregnation at the surface is not adequate for their further course to be followed. It seems, however, that they reach the graft from the granulation tissue which covers it at the surface of the brain (*c* in Text-fig. 2), and it is inferred that these fibres have grown in from the surface and that they are presumably derived either from the nerves in the dura mater, from vascular nerves, or possibly from nerves in the scalp tissues of the host.

The other set of regenerated nerve fibres is found in a deeply situated fasciculus of the grafted nerve (*b* in Text-fig. 2). Here a number (15–20) of stout, isolated fibres can be traced along the persistent endoneural sheaths in an upward direction, and at first inspection they appear to have taken their origin from the fibres of the adjacent part of the hippocampus. A closer study of the serial sections, however, shows no clear evidence for such a derivation. On the contrary, the fibres can be followed through a thin but apparently firm sheath of fibrous tissue, which here covers the grafted nerve bundle, into a small zone of granulation tissue at the adjacent margin of the lateral ventricle (*d* in Text-fig. 2 and Pl. 2, fig. 20). Here the fibres are collected into a circumscribed group which is intimately related to some newly formed blood vessels (Pl. 2, fig. 15). The origin of the latter is made quite clear by a consecutive inspection of the serial sections. The initial operation of implantation had involved a lesion extending deeply into the hippocampus. Through the track of this lesion a relatively large artery (*f* in Text-fig. 2) has extended up from the choroid plexus of the lateral ventricle towards the grafted tissue. It appears, indeed, that the regenerated

fibres just described have probably been brought to the graft by these invading blood vessels, and that they may therefore be derivatives of vascular nerves. However, though nerve fibres are certainly to be found in relation to the arteries of the choroid plexus, they are only indistinctly impregnated in these preparations, and it is not possible in the sections to trace them into continuity with those that enter the grafted tissue. Further, the regenerated fibres in their course downwards become intermingled with a plexus of hippocampal fibres which persist in the inflammatory tissue occupying the site of the lesion.

An intensive examination of every part of the grafted tissue, section by section, has failed to show any nerve fibre crossing directly either from the cortex or the white matter of the host brain into the nerve sheaths of the graft. Conversely, none of the regenerating fibres in the graft can be traced into direct continuity with fibres of the cortex or the white matter. The reaction of the nerve cells and fibres of the host brain in the proximity of the graft is similar to that in the previous experiments. That is to say, injured fibres are thickened, and show swollen retraction bulbs, irregular beading and so forth, while the cells of the cortex around the implanted tissue take on an intensive staining. At one point (at *g* in Text-fig. 2) the fibres of the white matter certainly assume an appearance of orientated growth, for they are arranged here in small compact bundles directed towards the graft (Pl. 2, fig. 13). At the margin of the latter they turn aside, suggesting that on reaching the perineural sheath they have become deflected from their original course. However, none of these fibres actually enters the grafted tissue, and a close study of the sections makes it probable that they are simply persistent remnants of pre-existing fibres which have become isolated and compressed into compact fasciculi by the cellular infiltration of the host tissue. Attention is further called to the fact that the host tissue near the graft has become infiltrated not only with macrophages, but also with fibrous elements. The disposition of these cell masses shows a slight but quite spurious resemblance to the Schwann cells and the macrophages of the nerve sheaths in the grafted tissue. Without the careful study of serial sections, it seems possible that this resemblance might lead to a misinterpretation, and that the persistent nerve fibres of the host brain which are found in such infiltrated zones might be mistaken for the ingrowth of new regenerating fibres into the grafted nerve. As already noted, however, it has not been possible to find any fibres from the cortex or white matter of the host brain directly entering the grafted tissue itself. Occasionally in the infiltrated white matter immediately adjacent to the grafted tissue a dichotomized fibre is to be seen, the branches swollen or beaded and perhaps ending in rounded and heavily impregnated expansions (Pl. 2, fig. 11). It is possible that such a disposition represents an abortive attempt at regeneration by sprouting on the part of the fibres. But it is equally possible (and in the absence of confirmatory evidence it seems probable) that the fibres are only the traumatized remains of pre-existing elements somewhat displaced from their normal position; for example, they may be surviving afferent cortical fibres which, on reaching the deepest layer of the cortex, normally break up into wide arborizations. It may be insisted again that, even if the appearance of some of these fibres might suggest to some histologists an attempted regeneration, in no instance could they be found to penetrate into the grafted tissue. Moreover, there is no reason to suppose that they may have been prevented from doing so by the barrier of the fibrous sheath surrounding the grafted nerve fragment. In Pl. 2, fig. 21, is shown a regenerating

fibre pursuing its course within the grafted nerve, while immediately adjacent (and separated by no definable barrier) are numerous fibres in the hippocampal alveus of the host. Yet these hippocampal fibres show no tendency to enter the grafted tissue.

The position of the grafted ganglion relative to the grafted sciatic nerve is indicated diagrammatically in Text-fig. 2. As already recorded, the shortest distance between the tissues of the two grafts is approximately 1·5 mm. The ganglion is firmly embedded in the cortex of the host and extends down to reach the subcortical white matter. Where it lies against cortical tissues it is covered by a firm fibrous capsule, though the latter in some places is very attenuated and consists of fine fibrous lamellae (Pl. 2, fig. 19). Most of the ganglion cells have undergone complete degeneration, to be replaced by proliferated satellite cells of which many persist in the characteristic form of residual nodules. However, seventy-three surviving ganglion cells were counted in the serial sections; they are arranged in a layer near the surface of the brain. The degenerated remains of the rootlets of the ganglion can be seen to extend towards the white matter from its deep aspect, and to bend up towards the surface of the cortex again. These rootlets consist mainly of endoneural sheaths with Schwann bands and many macrophages. Where they lie in direct contact with the subcortical white matter, the latter also is infiltrated with macrophage elements and there is no definite fibrous capsule between the grafted and host tissues at this point. Consequently it is difficult in some sections to determine precisely the boundary between the two.

Some of the surviving ganglion cells show the abnormal features which have before been described in transplanted ganglia, such as the outgrowth of multiple sprouts ending in globular enlargements and surface irregularities. Others have a normal appearance with a finely fibrillated cytoplasm, a well-defined central nucleus and a regular outline. Many fasciculi of newly regenerated fibres take origin from the cells. Of these some enter the adjacent capsule and can be seen extending along the interfaces of the fine lamellae of which it is here composed. Many of them end here in prominent end-bulbs, and none of them can be traced into the cortical tissue of the host. It is interesting to note, also, that the fibres of the cortical plexuses show no tendency to extend along the lamellae of the thin fibrous capsule which separates them from the grafted tissue, although these lamellae seem to provide the same opportunities for their regeneration as they do for the regenerating fibres of the grafted ganglion cells. In some sections the regenerated fibres of the graft and the fibres of the host cortex appear almost to come into contact with each other. Yet a study of the sections has failed to show that they intermingle at any point. Most of the regenerated fibres of the ganglion cells extend into the empty sheaths of the rootlets, and by sprouting some of them form a compact fasciculus which is enclosed in a single endoneural tube. Leashes of such fibres can be traced along the sheaths, in intimate relation to elongated strands of Schwann cytoplasm, until they approach the surface of the brain. Here they disappear from view in the sections. Others descend from the ganglion cells and then turn aside to reach residual nodules situated at a deeper level. These nodules form a conspicuous feature in many of the sections, and are rather better defined and more circumscribed than in the sections of Exp. 1. In some instances, the globular clusters of proliferated satellite cells are seen to be enclosed in the original mesothelial capsules of the atrophied ganglion cells. Many of the residual nodules are innervated by newly regenerated fibres which are derived from the outgrowing axones of adjacent ganglion cells. These fibres extend along narrow strands of Schwann cytoplasm, some-

times in fasciculi of three or four (Pl. 2, fig. 14). A study of the serial sections also makes it evident that the several fibres which innervate one residual nodule may sometimes be derived from more than one ganglion cell. At the point where the nerve fibres enter the nodule, the adjacent satellite cells stream out along them and appear to extend into continuity, or at least into series, with the cytoplasmic bands of the Schwann cells which conduct regenerated fibres to the nodule. Such an appearance accords well with the conception that the satellite cells related to each ganglion cell are morphologically (and perhaps functionally) equivalent to the Schwann cells of peripheral nerve fibres. It may be noted that some of the residual nodules receive their innervation from regenerated fibres which, after leaving a ganglion cell, creep along the surfaces of the fine lamellae of the fibrous capsule separating the grafted from the host tissue, and then enter the nodules on their superficial (capsular) aspect.

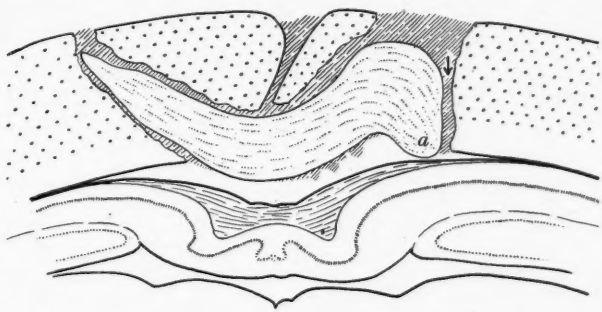
A general consideration of the grafted ganglion in this experiment leads to the conclusion that (as in Exp. 1) there has been vigorous regenerative activity on the part of the surviving ganglion cells, indicating that the grafted tissue provides all the essential facilities for the outgrowth of new nerve fibres. On the other hand, the most careful scrutiny of the sections gives no convincing evidence that the adjacent fibres in the cortex or white matter of the host brain avail themselves of these facilities. In other words, nowhere does there appear to be any regeneration or sprouting of new nerve fibres from the intrinsic fibres of the host brain. The implication of this negative statement is reinforced by the observation that at the deep aspect of the graft there appears histologically to be no capsular membrane separating the empty sheaths and the Schwann bands of the rootlets of the ganglion from the subjacent white matter of the host brain. Indeed, as already noted, it is rather difficult here to detect the precise boundary line between the grafted and the host tissue.

Exp. 8 (R. 192). Operation 5 May 1941, killed 19 May 1941. A small fasciculus of predegenerated sciatic nerve was implanted under the cortex, being introduced across the mid-line towards the opposite hemisphere immediately dorsal to the corpus callosum. The graft was taken from another animal in which the sciatic nerve had been cut 14 days previously on 21 April.

Serial sections of the implantation site were prepared by the pyridine-silver method. They show that the graft has not taken up the position originally intended; the fasciculus extends obliquely through the cortex and subjacent white matter of one hemisphere, reaching from the surface of the brain down to the corpus callosum in the mid-line. The tissue is firmly encapsulated by a layer of fibrous tissue except where its cut end lies in the white matter. The endoneural sheaths are filled with macrophages, and in some parts of the graft the Schwann bands are conspicuous and appear to be in a healthy condition. There are no signs of the ingrowth of newly regenerating fibres. The tissue of the host has been sharply impregnated, and many traumatized fibres are to be seen in the neighbourhood of the graft. On the other hand, there is no histological appearance which could be interpreted as evidence of regenerative processes.

Exp. 9 (R. 191). Operation 5 May 1941, killed 19 May 1941. A small fragment of predegenerated sciatic nerve was implanted under the cortex, extending across the mid-line from one hemisphere to the other in the position of the corpus callosum. The graft was taken from another animal in which the sciatic nerve had been cut 14 days previously, on 21 April.

Serial sections of the implantation site were prepared by the protargol technique. The grafted fasciculus is seen to occupy the position shown in Text-fig. 3. There has been comparatively little reaction in the surrounding tissue of the host brain, and there is no dense fibrous capsule such as that found round the implanted tissue in some of the other experiments. The grafted fasciculus reaches down through the subcortical white matter as far as the roof of the lateral ventricle, and at one point it becomes adherent to the dorsal surface of the hippocampal formation by a layer of granulation tissue. The graft seems to be mainly vascularized by new vessels which have extended in from the surface of the brain. It consists for the greater part of endoneural tubes packed with macrophages in characteristic fashion. Here and there are to be seen Schwann cells, but nowhere are these arranged in regular formations of Büngner's bands. A careful search through the serial sections shows no evidence of newly regenerating nerve fibres in the graft except at a point where one end of the fasciculus reaches the cavity of the lateral ventricle. In this region (*a* in Text-fig. 3) about 6–10 stout nerve fibres can be seen growing separately along the endoneural sheaths of the graft. One of them is shown in Pl. 3, fig. 24. They extend only a short



Text-fig. 3. Diagram showing the position of the grafted nerve fragment in Exp. 9.

distance, soon ending in irregular thickenings and terminal branchings. They are undoubtedly newly formed axones which have penetrated into the grafted tissue. On being traced to their source by following the serial preparations section by section, they are eventually found to penetrate the thin lamella of fibrous tissue which covers the graft at this point, and to enter an adjacent zone of granulation tissue. Here it becomes evident that the sprouting axones are related to a vascular invasion of the grafted tissue, for they are seen to form a compact fasciculus running alongside a leash of small blood vessels which are present in the granulation tissue and from which branches extend into the grafted nerve. The appearance at this level is very similar to that described in Exp. 7, and illustrated in Pl. 2, fig. 15. For a further distance, the nerve fibres can be traced towards the surface of the brain, but here they become lost in the densely stained inflammatory tissue which occupies the site of the operative lesion. From these observations, the possibility may be inferred that vessels extending through the cortex from the surface in order to vascularize the graft (in the position marked by an arrow in Text-fig. 3) have carried with them a small fasciculus of nerve fibres, and that it is from the latter that some axones have actually penetrated into the graft in the course of their regenerative activity. Certainly, the serial sections

provide no evidence that the regenerating fibres in the graft have been derived from the cerebral neurones of the host brain. Indeed, it was not possible to find any indication that the implantation of the predegenerated fragment of sciatic nerve had induced regenerative processes in the intrinsic nerve fibres of the host brain. It should perhaps be again emphasized that in this experiment there has been no formation of a fibrous capsule around the graft whose density might serve to impede the ingress of newly sprouting fibres if the latter had actually been present in the nervous tissue of the host brain. At least, the histological appearance does not provide evidence of such a barrier.

Exp. 10 (R. 189). Operation 5 May 1941, killed 19 May 1941. A small fragment of predegenerated sciatic nerve was implanted under the cortex, extending across the mid-line from one hemisphere to the other immediately dorsal to the corpus callosum. The graft was removed from another animal in which the sciatic nerve had been cut 14 days previously, on 21 April.

Serial sections of the implantation site were prepared by the pyridine-silver technique. They show the graft firmly embedded in the cortex. It is covered by a thin but dense fibrous capsule, except at one extremity where the cut ends of the endoneural sheaths abut against the host cortex with no organized capsule intervening. In the deeper part of the graft, many healthy Schwann elements are present in closely arranged protoplasmic strands—typical Büngner's bands. Elsewhere but few Schwann cells seem to have survived, and the endoneural tubes are packed with macrophages among which are frequently to be seen degenerated remains of axones. Nowhere in the grafted nerve tissue are there any regenerating nerve fibres, even at that point where there appears histologically to be no definite fibrous layer which might form a mechanical barrier to the ingress of newly growing fibres from the host tissue if these had been present. The axones and cell processes of the white matter and cortex of the host brain are very well impregnated. In the immediate neighbourhood of the implanted tissue many of the traumatized fibres of the host brain show degenerative changes, such as irregular beading, thickenings, and retraction bulbs. On the other hand, the histological appearance provides no evidence of regenerative processes. Certainly no sign of the sprouting of new fibres can be found anywhere in the serial sections.

Exp. 11 (R. 196). Operation 20 May 1941, killed 6 June 1941. A small fasciculus of predegenerated sciatic nerve was implanted longitudinally beneath the surface of the cortex of the left cerebral hemisphere. The graft was taken from another animal in which the sciatic nerve had been sectioned 10 days previously, on 10 May.

Serial sections, treated by the pyridine-silver method, show that the implanted fragment lies well buried in the cortex, extending into the subjacent layer of white matter. Its deep surface is in close contact with the roof of the lateral ventricle. It is everywhere surrounded by a firm fibrous capsule. Within the graft the endoneural sheaths of the degenerated nerve fibres are filled with vacuolated macrophages, and there is little evidence of band formation by the Schwann cells. Here and there, however, they are present as isolated fusiform strands. No regenerated axones are to be found anywhere in the graft. In the surrounding brain tissue the impregnation has been particularly successful, and the nerve fibres are sharply delineated. While those in the immediate vicinity of the graft show many degenerative changes, such as varicosities, terminal expansions and globular bulbs, no evidence of regeneration

can be detected. Neither in the cortex nor in the white matter is there any indication of the outgrowth of new fibres, or of a tendency for the fibres to orientate themselves in the direction of the graft. There is little inflammatory reaction in the cortex, but in the adjacent white matter there is a rather dense cellular infiltration.

Exp. 12 (R. 197). Operation 20 May 1941, killed 7 June 1941. A small fasciculus of predegenerated sciatic nerve was implanted longitudinally beneath the surface of the cortex of the left hemisphere. The graft was taken from another rabbit in which the sciatic nerve had been sectioned 10 days previously, 10 May.

When the animal was killed, the graft was found lying on the cut surface of the brain and only loosely attached to the underlying tissue. Serial sections were cut through the site of the implantation and treated by the pyridine-silver technique. They show some inflammatory reaction in the superficial part of the cortex, with a mild degree of necrosis. This region is infiltrated with macrophages and contains dilated blood vessels. In the midst of the inflammatory tissue there remain many injured nerve fibres which stain intensely black and often show an irregular beading, and fusiform or rounded swellings at their free ends. At some points the cellular infiltration has displaced and separated the components of the fibre plexus in the cortex, compressing them into leash formations which, on superficial inspection, might be mistaken for an orientated growth of new fibres. It should also be noted that the necrotic tissue of the cortex is to be distinguished clearly from possible remnants of the nerve graft, none of which, however, are actually present in the sections.

Exp. 13 (R. 122). Operation 5 June 1940, killed 19 June 1940. In this experiment, the proliferated Schwann tissue from the peripheral stump of a cut tibial nerve was implanted in the cortex of the left hemisphere. The tibial nerve (of another animal) had been cut a little over 7 weeks previously, on 12 April, and 2 cm. of the trunk had been resected. There had been no reunion. The Schwann outgrowth from the peripheral stump was snipped off and the fragment placed in an incision in the cortex.

Serial transverse sections of the implantation site were prepared with the protargol technique. They show the grafted tissue embedded in the cortex with the free end of the original stump extending down into the subjacent white matter. The superficial part of the implanted fragment, which is covered by its original sheath of epineurium, is composed of slender Schwann elements aligned in a longitudinal direction and interspersed with macrophages. In contrast with the grafted nerve fragments of the other experiments, the implanted tissue in the present case contains no framework of well-defined endoneural tubes; it consists mainly of newly formed tissue resulting from proliferation at the peripheral stump. The Schwann cells in the main part of the graft are drawn out into attenuated fibrils which often extend for a considerable distance, giving rise to a loose fibrillary structure which somewhat resembles collagenous tissue. In the more deeply situated part of the graft, however, the Schwann cells are aggregated into a compact bunch of nucleated cytoplasmic strands (Pl. 3, fig. 25). Moreover, the cells are evidently undergoing active proliferation, for mitotic figures are to be seen here and there. Indeed, it is clear that subsequent to the implantation there has been considerable growth of the cellular elements of the 'Schwannoma' of the peripheral stump. Further, many of these proliferating elements have penetrated into the immediately adjacent zone of the host brain, for strands of elongated cells which have the general appearance of Schwann cells are found dispersed somewhat irregularly in the granulation tissue surrounding the stump of the original implant. It will be

realized, therefore, that it is hardly possible in this area to determine exactly the limits between host and grafted tissue.

A search through the serial sections shows that, in relation to the invading strands of Schwann cells, there are some slender leashes of nerve fibres which are quite certainly undergoing regeneration (Pl. 3, figs. 26, 29). They are deeply impregnated, show irregular beaded swellings along their course (particularly at points of dichotomization), and send off numerous collaterals. Of the latter, many pursue a parallel course while some turn in a recurrent direction. Moreover, these fibres have the appearance in some sections of clinging to the surface of the Schwann strands, and of extending along them in a characteristic manner. While it is possible to affirm the presence of these newly regenerating fibres related to Schwann elements derived from the grafted tissue, serious difficulties arise when an attempt is made to follow them to their source. They can be traced through the serial sections into the granulation tissue which infiltrates the adjacent region of the host brain, but here they become intermingled inextricably with numerous fibres of the white matter which persist amid the cellular infiltration. Indeed, on first inspection the appearance suggests that the new fibres have certainly regenerated from fibres of the white matter of the host brain. However, an alternative interpretation requires consideration; the granulation tissue is clearly in process of vascularization by the ingrowth of new blood vessels, and it remains possible that the new fibres may have been introduced in association with these. On the other hand, unlike the preparations in Exps. 7 and 9, no direct evidence was found of a circumscribed fasciculus of nerve fibres accompanying a localized group of invading blood vessels towards the graft. If strands of regenerating fibres are followed towards the graft they are found to break up into fine terminal fibres and to lose themselves in the proliferating mass of Schwann cells which issue from the tip of the grafted fragment. A careful search showed only one clearly demonstrable nerve fibre actually within the original confines of the grafted tissue. That is to say, with this exception, the regenerating fibres appear to extend only into relation with the Schwann cells which have grown out from the graft after implantation. The exception referred to is an exceedingly slender fibre, with a few varicose swellings, which is seen to extend along one of the elongated Schwann cells in a single section (no. 71·4 of the series). Unfortunately, the protargol staining is not sufficiently selective to allow certain identification of such a fibre except where it is cut along its length. Consequently this fine regenerating fibre cannot be picked up again in adjacent sections, and it is not possible therefore to trace its course.

If it is a fact that the few newly sprouting nerve fibres in the neighbourhood of the graft are derived from the axones of the white matter of the host brain, then it is clear that an extremely small—indeed almost insignificant—proportion of these axones have undergone any regenerative process. Multitudes of fibres in the white matter adjacent to the graft, which have been affected directly or indirectly by the implantation lesion, show no histological evidence of regeneration. On the other hand, many show the usual signs of traumatic reaction such as irregular thickening, retraction bulbs and so forth. Yet a general view of some of the sections gives the impression of fibres of the white matter converging in all directions towards the Schwann proliferation at the tip of the grafted fragment, and it is clear that this appearance might be interpreted as striking evidence of a powerful outgrowth of regenerating fibres, 'attracted' in some way by elements in the implanted tissue. Such a conclusion is

further strengthened by the presence of small and rather compact bundles of fibres which can be seen to emerge from the main feltwork of white matter and to pass towards a thin capsular layer of fibrous elements separating the white matter from the proliferating mass of Schwann cells (Pl. 3, figs. 22, 23). Some of these fasciculi abruptly end at the margin of the capsule. Others turn aside when they reach the fibrous capsule, and their component fibres seem to run over its surface or to commence a recurrent course. In this behaviour they closely resemble the similar bundles described in Exp. 7, and illustrated in Pl. 2, fig. 13. Some fasciculi, however, actually enter the fibrous capsule and their fibres either end abruptly here or turn aside almost at a right angle to extend between the elongated and flattened cells of the capsule. Thus their disposition suggests that they are regenerating fibres which are actively pushing their way towards the grafted tissue but which have been diverted from their objective by the mechanical barrier of the fibrous capsule. However, a closer inspection suggests an alternative interpretation. In the first place it should be noted that none of these fibres of the white matter which are orientated towards the graft in compact fasciculi can be found actually to enter the original confines of the grafted fragment. Secondly, it is apparent that the converging appearance is at least partly the result of the mechanical traction exerted by the grafted tissue on the surrounding brain tissue of the host. This traction effect is made very evident by the formation in the immediately adjacent part of the roof of the lateral ventricle of a recess which has been pulled up towards the graft. In other words, the pre-existing fibres of the white matter in this region (or what remains of them) have become to some extent secondarily distorted, and, so far as this has occurred, there is clearly no need to postulate the orientated growth of new fibres to explain their disposition in respect of the graft. Lastly, the fibres of the converging fasciculi do not *individually* show distinctive morphological features which conclusively indicate that they are actively regenerating fibres. In this respect, they are very different from the regenerating fibres in relation to the invading strands of Schwann cells described above, for they do not present the characteristic beading and dichotomization. The possibility must be entertained that, where small groups of such converging fibres appear to penetrate a capsule of fibrous elements to extend towards the graft, this appearance may perhaps be explained by the fact that a secondary capsule of fibrous elements is in process of formation by the glial tissue of the host in reaction to the infiltration of the brain by granulation tissue and proliferating Schwann elements. Some of the pre-existing fibres of white matter have become 'caught up' in this capsular formation and thus appear to be in the process of actively penetrating it. Others may have become pushed aside by the cellular proliferation so that they appear to be diverted from their presumed outgrowth towards the graft. Yet it is difficult to arrive at a decision on this matter, for some of the fibres of the converging fasciculi which penetrate the secondary capsule become intermingled with the undoubtedly regenerating fibres which have been described above as extending along infiltrating Schwann elements. Unfortunately, it is not possible to be absolutely certain whether these two sets of fibres are actually continuous with each other.

DISCUSSION

The records of our experimental results, detailed above, together with the evidence of the photomicrographs which illustrate them, bear adequate testimony to the accuracy of Tello's observations. There can be no doubt at all that the implantation of predegenerated nerve fragments in the brain is accompanied by the appearance of regenerating fibres. As already noted, however, certain difficulties arise in the interpretation of some of the histological pictures, and in determining the precise origin of the regenerating fibres.

In ten experiments, portions of predegenerated sciatic nerve were successfully implanted in the cerebral hemisphere. Serial sections showed that in these cases the grafts had become firmly embedded in the cortex and subjacent white matter, with a survival in varying degrees of the Schwann cells. In some experiments the latter were found to be disposed in characteristic alignment, producing the appearance of 'Büngner's bands'; in others it was clear that most of the cells had disappeared, for only scattered isolated elements could be seen. In three of the experiments (7, 9 and 13) newly regenerating axones were found actually inside the grafted fragment, extending for some distance within the endoneural tubes. These axones present all the characteristic morphological features of actively growing fibres, such as beading, dichotomization, thickening at the origin of collaterals, the presence of several fibres within one endoneural sheath, the persistence of remains of the old degenerated axone in the same sheath as the new axone, and so forth. They are, indeed, quite similar in appearance to those figured by Tello in Fig. 1 of his monograph. But the most certain evidence that they are in active process of regeneration is provided by their position inside the endoneural tubes of the implanted tissue, at a time when all the original fibres of the grafted nerve have entirely disappeared except for scattered granular debris. The experiments demonstrate beyond doubt, therefore, that implanted fragments of predegenerated nerve may lead to the appearance of regenerating axones within the brain of the host. The source of these new axones is not so readily determined, for it cannot be accepted without further consideration that they are actually derived from the intrinsic fibres of the brain. Other possible sources are (*a*) from the surface of the brain, extending in here either from nerve fibres in the meninges or from fibres in the tissues of the scalp which overly the skull defect, or (*b*) from vascular nerves accompanying either the vessels which penetrate from the surface of the brain or from the choroid plexuses. In regard to these alternatives, reference may be made to the observations of S. L. Clark (1929, 1931, 1934) that the vessels within the substance of the human brain and spinal cord, as well as those in the pial tissues on the surface and in the choroid plexuses, are accompanied by leashes of fibres, both myelinated and unmyelinated. The same author has also demonstrated that there are considerable numbers of nerve fibres coursing in the pial tissue and entirely unrelated to blood vessels. Chorobski & Penfield (1932) showed that in the monkey's brain there may be as many as 8–12 vascular fibres accompanying the intracerebral arteries. Lastly, Stöhr (1938) has demonstrated and figured rich plexuses of nerve fibres related to vessels in the pia mater and choroid plexus, as well as rather complicated 'sensory endings' in the pial tissues. Thus, apart from the intrinsic fibres of the central nervous system, there appear to be abundant sources for the regeneration of extrinsic fibres in relation to tissues implanted within the cranial cavity. In relation to this, it should

be emphasized that in the three experiments in which regenerating fibres were undoubtedly present in the grafted nerve fragment, they were extremely few in number. Thus in Exp. 7, not more than thirty fibres were counted, in Exp. 9 not more than ten, while in Exp. 13 one fibre only was found within the original confines of the graft.

It has not been possible to determine with certainty the source of the newly formed fibres in these three experiments. In two cases, however, by following them in consecutive serial sections they were traced through the fibrous sheath of the implanted nerve fragment into an adjacent zone of granulation tissue. Here they were found to be collected into fairly compact fasciculi in immediate relation to invading blood vessels. This appearance suggests that the regenerating fibres may have grown out from vascular nerves, extending along the blood vessels which vascularize the graft. On the other hand, the possibility has not been excluded that they may have been derived from intrinsic fibres of the host brain, which have taken advantage of invading blood vessels to reach the graft. For outside the graft they become so closely intermingled with persisting fibres of the cortex or white matter, in amongst which the sprouting vessels push their way, that it is here no longer feasible to trace them in continuity from section to section.

Against the suggestion that the regenerating fibres are vascular nerves is the fact that their size and number seem to be out of all proportion to the size of the small blood vessels with which they run in association. It must also be noted that, in the normal tissues of the host brain which have not been obviously affected by the proximity of the graft, the vascular nerve fibres are not certainly demonstrable by the pyridine-silver and protargol methods employed. However, it remains possible that they are more readily and deeply impregnated with silver in the traumatized tissues (as is certainly the case with the intrinsic fibre plexuses of the brain) and hence become much more easily demonstrable here. The suggestion obviously presents itself that the question of the possible vascular origin of the regenerated nerve fibres in such experiments as these might be determined by the subsequent section of the cervical sympathetic cord. This procedure is unlikely to be decisive, however, for Chorobski & Penfield (1932) have shown that, in the monkey's brain, a large number of vascular nerves remain intact even after destruction of the sympathetic and known parasympathetic sources from which they might be derived. In Exp. 7, some of the regenerating fibres within the grafted nerves were traced up to the surface of the brain, and the appearance here suggests that they have grown in from nerves in the meninges, or even from the tissues of the scalp. Lastly, in Exp. 13 the single nerve fibre found within the grafted fragment could not be traced into the surrounding tissue.

In only one experiment, no. 13, were undoubted regenerating fibres identified in the tissue immediately surrounding the grafted nerve fragment. In this case they were intimately associated with strands of Schwann cells which had sprouted out from the graft to invade the adjacent brain tissue of the host. Under a high power they are quite similar in appearance to those figured by Tello in Fig. 3*f* of his monograph. The precise origin of these regenerating fibres presents the same difficulties as those found inside the grafts. That is to say, when traced into the white matter of the host brain, they become lost among intrinsic nerve fibres. However, the general appearance in the serial sections is somewhat more persuasive that they are actual derivatives of the fibres of the white matter, for they are not found to associate themselves closely with individual blood vessels which invade the tissues surrounding the graft. Never-

theless this does not exclude the possibility of their origin from extrinsic fibres such as those which are known normally to accompany intracerebral vessels.

In some of the experiments, particularly nos. 7 and 13, the fibres in the white matter immediately adjacent to the grafted nerve fragment were found to be disposed in a definite orientation towards the latter. Compact fasciculi appear to emerge from the white matter and to penetrate into a secondary capsule formed in the host tissue around the graft. This arrangement has been described by Tello, and accurately illustrated in his Figs. 2 and 3. He regards it as strong evidence in favour of a chemotropic influence exerted by the grafted tissues on the fibres of the host brain. He further develops this conception by describing the appearance of fibres which seem to be 'seeking' a vulnerable point in the ensheathing capsule in order to enter the graft, some taking advantage of the path offered by blood vessels, others turning back again after determined but unsuccessful efforts to reach their objective. In our own experiments, however, it seems doubtful whether it is necessary to explain the histological appearance on the basis of such behaviour, for, as already noted in the description of Exp. 13, there is no certainty that the orientated fibres are really in process of regeneration. None of them are actually found to enter the grafted fragment of nerve, and the fibres do not individually show morphological features which can be regarded as indubitable evidence of active growth. On the other hand, it is possible that they are pre-existing fibres whose disposition has been secondarily disturbed (1) by the mechanical traction on the surrounding tissues exerted by the implanted fragment,[1] (2) by the mechanical effect of a cellular infiltration of the white matter which has led to the compression of many of the fibres into compact fasciculi, and also to some distortion of their original course, and (3) by the formation of a secondary capsule of fibrous elements round the implanted tissue, in which fibres of the white matter have become caught up so as to give the impression of active penetration, and which in some cases has pushed the fibres to one side so as to give the impression that the latter have diverged from their original path in order to 'seek' a favourable point of entry into the graft. A careful study of the actual sections makes it seem very probable that these factors are almost certainly responsible for the histological appearances; certainly it is not legitimate to conclude that the latter betoken a process of active regeneration unless more convincing evidence is forthcoming. This question is clearly of great importance for, as already noted, the appearance in the brain of converging fasciculi of fibres directed towards an implanted nerve fragment has been advanced as a proof of the potency of the peripheral stump of a cut nerve to stimulate and attract by chemotropic influences the outgrowth of new axones. Possibly the problem could be solved by a more extended series of experiments allowing much longer time intervals for the process of regeneration (if it really occurs) to express itself in a more convincing histological picture. If, for example, these converging fibres were found actually to enter the original confines of the grafted fragment, then there could be little doubt that they must have done so by their own growth. In this connexion, we may note that Tello's figures also show that these converging fasciculi do not penetrate the grafted nerve, but stop abruptly at the surface of its fibrous capsule, or become diverted and bent back at this level. Again, if it could be demonstrated that no type of grafted

[1] The appearance of the sections in Exp. 13 recalls the experiments of Weiss (1934) demonstrating the traction effect of proliferating masses of cells in tissue cultures which, according to this author, is the result of a local dehydration of the surrounding culture medium.

tissue other than nerve fragments with active Schwann elements is capable of producing a similar histological appearance, this again would provide strong support for Tello's interpretation.

The implantations of spinal ganglia were not successful in so far as they were designed to show whether the adjacent grafted nerve fragment exercised any orientating influence on the outgrowth of fibres from the surviving ganglion cells. In the one experiment (no. 7) in which both the ganglion and the nerve fragment remained in situ in active condition, the disposition of the endoneural sheaths in the attached rootlets of the ganglion directed the regenerating fibres of the ganglion cells upwards towards the surface of the brain, and thus the situation was hardly favourable for determining whether the fibres might be attracted towards the grafted nerve fragment. However, the outgrowth of processes from the ganglion cells in Exps. 1 and 7 provided the opportunity for a number of instructive observations. In the first place, the regenerative process appears to be very vigorous. Axonal fibres stream down the endoneural sheaths of the rootlets, and numerous fibres from surviving ganglion cells grow out to residual nodules in the substance of which they spin rich plexuses. The outgrowth of abnormal processes from the surviving ganglion cells may be taken as a further indication of their growth activity, though these can hardly be called regenerative in the proper sense. It is perhaps better, using Nageotte's nomenclature (1907), to term such outgrowths 'paraphytes' to distinguish them from the dendritic processes of normal multipolar cells, or 'orthophytes'. In any case, the ganglion cells appear to be transformed into multipolar cells. Ranson (1914) reported that this is only a temporary phase, for he found that after a time the cells of grafted ganglia return to their original unipolar form. This observation was confirmed later by Tidd (1932) who found nerve cells of normal appearance still persisting in a spinal ganglion which had been grafted in the brain 99 days previously.

It appears certain, then, that the environment of the brain does not of itself offer any hindrance to the regeneration of nerve fibres provided that there are neuronal elements capable of regeneration and provided adequate paths for regeneration are available. The remarkable 'neurotization' of residual nodules has been supposed by Nageotte and Cajal to be the result of a chemotropic influence exercised by the satellite cells which compose them. It appears from our own material, however, that nerve fibres reach the nodules by extending along strands of Schwann cells which run into continuity with them. If the cells of the residual nodules are indeed chemotropically active in relation to the regeneration of nerve fibres, they do not appear to exert any such influence on the nerve fibres of the host brain which lie in close relation to them, in some cases separated only by a relatively narrow zone of loosely packed round cells. In no instance were any fibres of the host brain observed to extend out towards a residual nodule in the grafted tissue. It may be noted here that in Exp. 7, outgrowing processes from the ganglion cells were observed to reach the surface of the ganglion and to be extending between the fine lamellae of the connective tissue capsule which separates the graft from the tissue of the host brain. Some of these fibres run into the ganglion again to approach residual nodules from the periphery. Presumably, similar paths were therefore available for the extension of regenerating fibres from the host brain had these been present.

Sprouting nerve fibres are in some sections seen to extend from the ganglion cells into the zone of cellular infiltration separating the host cortex from the graft—demon-

strating that this zone is pervious to regenerating fibres (see Pl. 2, fig. 16). Yet in no case was it possible to demonstrate regenerating fibres proceeding from the host tissues in the reverse direction, even though (as shown in Pl. 3, fig. 27) invading blood vessels extending into the graft across this zone seem to provide a ready pathway for their extension.

Attention may be drawn to further evidence of the inactivity of the intrinsic fibres of the host brain in close proximity to grafted nerve tissue because, although this evidence is of a negative nature, it is believed to be important for assessing the regenerative capacity of cerebral neurones. In many regions the graft is separated from the host tissue by a layer of comparatively dense fibrous tissue which may seem to provide an impenetrable barrier to regenerating fibres. On the other hand, as recorded in the detailed descriptions of the experiments, in some places the endoneural tubes of the grafted nerve, with their contained Schwann elements, appear histologically to be separated from the host tissue by no definite fibrous layer which might prevent the ingress of new fibres. This is the case in Exp. 7, for example, in which the Schwann cells are apparently active and vigorous. Indeed, that they are able to play their normal role in facilitating the growth of regenerating fibres is demonstrated by the fact that a few such fibres are actually present in the graft, though their origin could not be traced directly to the intrinsic fibres of the host brain. Yet at one point where the endoneural tubes and their contained Schwann elements are in direct contact with the white matter of the host brain, separated by no intervening tissue, the fibres of the white matter can be seen at a distance of less than 20 μ from a regenerating axone which has descended from the surface of the graft. Even here the fibres of the host brain show no tendency to invade the grafted tissue.

These observations clearly lead to the conclusion that if the intrinsic nerve fibres of the brain possess any regenerative capacity it must be extremely feeble in comparison with that of fibres in peripheral nerves. On the other hand, the series of experiments has certainly demonstrated that implanted fragments of predegenerated nerve may be associated with the outgrowth of new nerve fibres within the brain of the host. Even though these regenerating fibres are few and their precise origin uncertain, they do provide definite evidence that the process of regeneration is in some manner facilitated by the presence of the grafted tissue, for in our experience (and using the same histological techniques) such regenerating fibres are not seen in the brain of rabbits or other mammals following ordinary experimental lesions of various kinds. Our experiments further suggest that the facilitating agent is to be found in the Schwann elements rather than the endoneural tissue, for the most vigorous of the few regenerating fibres which have penetrated into the grafted nerve fragment are found in those parts of the graft where the Schwann elements appear particularly well preserved. Even more suggestive of this interpretation is the appearance of fasciculi of regenerating fibres in Exp. 13, in intimate relation to proliferating strands of Schwann elements which have infiltrated the brain tissue of the host. Lastly, attention may again be drawn to the fibres in Exp. 7 (of which one is illustrated in Pl. 2, fig. 20) which are seen to penetrate what histologically appears to be a fairly dense barrier of fibrous tissue in order to gain the interior of the grafted nerve, for the histological picture in this case does suggest that the penetration is in some manner facilitated by the influence of the tissues within the graft. Clearly, however, it would be desirable before reaching any final conclusion on this matter to ascertain the effect

of other kinds of tissue implanted in the substance of the brain. Reference may be made in this connexion to a paper by Shirai (1935) in which is described the results of the implantation of portions of skin into the brain of rabbits. According to this author, the axones of the brain showed evidence of active regeneration 7 days after the operation, and this process reached a maximum after an interval of 50 days. Indeed, he concludes that the regenerative capacity of fibres in the brain is not inferior to that of peripheral nerve fibres. He notes particularly that the newly growing fibres show a tendency to extend along the blood vessels which run from the tissues of the host brain to penetrate the implanted skin, and that they also extend along strands of epidermal cells which have proliferated from the grafted tissue. However, it is not quite clear from the descriptions how far it was possible to define accurately the boundary line between the original graft and the tissue of the host brain so as to be certain that nerve fibres seen among epidermal cells have actually reached this position by their own growth, and that they are not merely pre-existing fibres of the white matter which have been surrounded by the proliferation of epidermal elements subsequent to implantation. The author also does not indicate precisely the morphological criteria by reference to which he determines that the nerve fibres referred to are really in process of active regeneration. In any case, however, even if Shirai's contention that the implantation of fragments of skin in the brain leads to the regeneration of nerve fibres is correct, the regeneration may be attributable to associated elements such as the Schwann cells of the cutaneous nerve plexuses.

In 1928, Oiye reported on an extensive series of experiments in which different foreign bodies were implanted in the brains of rabbits. He was able to persuade himself that new outgrowths of cerebral axones occurred in relation to the implanted materials 21 days after operation. For example, in the case of porous pieces of celloidin, numerous axones were found penetrating into the pores. It is stated that, even 3 years after operation, the axones of the brain tissue still showed evidence of active regeneration. However, it is not easy to assess the validity of these statements, for the paper is illustrated by extremely poor photomicrographs which fail to supply convincing evidence in support of the descriptive account. The author also fails to take account of the passive displacement of pre-existing fibres in the host brain as the result of contraction of the tissues in the neighbourhood of the graft. Indeed, it appears quite certain that one of his figures (Pl. 6, fig. 10), which purports to show a massive number of axones penetrating into the pore of a celloidin fragment, simply illustrates the distortion effected on the adjacent white matter of the brain by the presence of a foreign body. It may be remarked that, if Oiye's experiments should be confirmed, they indicate that the regenerative phenomena reported by Tello in his experiments on the implantation of elder pith soaked in saline extract of a degenerating sciatic nerve are not necessarily dependent on a neurotropic principle derived from the Schwann cells.

It is pertinent, in discussing the reaction of cerebral neurones to grafted tissues, to refer to experiments carried out recently in this laboratory in which fragments of foetal cerebral cortex were implanted in the brain of young rabbits (Le Gros Clark, 1940). It was noted in this material that there was very little reaction in the brain of the host to these implantations, even though the foetal tissue underwent considerable proliferation and differentiation. Certainly there was no evidence of any regeneration of the fibres of the host brain in the neighbourhood of the grafts.

It is not proposed to attempt here a complete survey of the literature bearing on the regenerative capacity of neurones in the central nervous system of mammals. However, certain references other than those already mentioned require attention because of their general pertinence to the present study. Cajal (1928) has described abortive regenerative phenomena in the spinal cord, particularly of young animals. Dustin (1910) implanted segments of predegenerated nerves in the spinal cord of guinea-pigs and reported some regenerative activity, particularly of the fine fibres, which appeared to be arrested by the connective tissue of the cicatrix. He also states that fibres of the posterior spinal roots penetrated into the scar and often divided into ascending and descending branches. In none of his experiments, however, did he find regenerating fibres, either from the posterior roots or from the spinal cord itself, penetrating into the grafted nerve fragment. He concludes that the absence of regeneration in the central nervous system under ordinary conditions is due partly to the lack of suitable pathways which would serve his odogenetic principle, and partly to the fact that the capacity for growth is much more limited in the neurones of the central nervous system than in peripheral nerves. Ranson (1903) observed newly grown medullated fibres crossing the line of a lesion made in the cerebral cortex of young rats. He concluded, however, that these were not examples of true regeneration, but were simply the product of neurones still immature at the time of operation, for he found the number of new fibres in the scar to diminish regularly as the age of the operated animal increased. It was later shown by Allen (1912) that in the rat cerebrum mitosis continues with a considerable degree of activity up to the 29th day after birth. Ranson found, indeed, that in his young rats the nerve fibres, instead of possessing any unusual regenerative power which might be expected at this stage of immaturity, tend to degenerate completely after injury.

Of more recent work on regeneration in the central nervous system, reference may be made to the work of Migliavacca (1930) and Sugar & Gerard (1940). The first of these reported evidence of regeneration after section of the spinal cord in foetal and new-born rats. It is well recognized, however, that the central nervous system of the new-born rat is very immature, and it is possible that any post-operative growth of new fibres may therefore be due, not to regeneration in the proper sense, but to the developmental outgrowth of axones which would be proceeding normally at this time. The work of Sugar & Gerard suggests more interesting possibilities, for they sectioned the spinal cord in young rats aged 3–5 weeks, and in some cases also reported regeneration across the scar. Their anatomical evidence, however, is not easy to assess, for the appearances shown in the photomicrographs illustrating their paper are hardly decisive enough to carry final conviction. It may be pointed out, also, that the consistency of the white matter of the central nervous system often makes it difficult to avoid a slight degree of pulling and tearing in attempts to produce a perfectly clean transection experimentally. Thus, some of the fibres which appear under the microscope to cross the scar at the site of transection may possibly be the frayed ends of pre-existing fibre bundles pulled out of their normal position and left overlapping the line of the knife-cut. Lastly, the relative immaturity of the central nervous system of young rats even a few weeks old requires to be taken into account in these experiments. According to Allen, mitosis actually increases in the spinal cord of white rats for a time after birth, reaching its high point at about the 7th day, but no mitoses are found after the 18th day. On the other hand, the developmental outgrowth of new

axones may be presumed to be continued for some time after this. Indeed, Allen states that complete cellular differentiation in the wall of the central canal is accomplished in the lumbar region only by the 30th day. It remains possible, therefore, that any new fibres observed by Sugar & Gerard in their experiments may be the result, not of true regeneration, but of the outgrowth of processes derived from neurones still immature at the time of operation.

SUMMARY

1. Fragments of predegenerated sciatic nerve implanted in the brain were penetrated by regenerating nerve fibres in three experiments.

2. The regenerating fibres found within the grafts were very few in number.

3. In two cases, these regenerating nerve fibres were traced to a fasciculus accompanying newly formed blood vessels which approached the graft from the tissues of the host brain. In one case the regenerating fibres within the grafted nerve fragment appeared to have entered the latter from the tissues at the surface of the brain.

4. In no case could the regenerating fibres be traced with certainty into continuity with the intrinsic fibres of the host brain, though the possibility of such a derivation is not excluded.

5. In some cases the endoneural sheaths of the grafted nerve, with their associated Schwann cells, were found to be in direct contact with the white matter of the host brain, with no histological evidence of an intervening layer of fibrous tissue. Yet at such points the fibres of the white matter show no tendency to invade the grafted tissues.

6. In some experiments many fibres in the surrounding white matter were found to be disposed in fasciculi orientated towards the graft. This appearance may be due partly to a traction effect imposed on the host tissue by the graft, and partly to the dislocation of pre-existing fibres by a cellular infiltration.

7. In one experiment, proliferating strands of Schwann cells had invaded the tissues of the host brain. Associated with these strands were found some undoubted regenerating fibres. Their precise origin, however, could not be determined.

8. In two experiments, spinal ganglia were grafted successfully in the brain. The surviving ganglion cells showed great regenerative activity. Many fibres were found to penetrate into a zone of cellular infiltration separating the graft from the tissues of the host brain. On the contrary, no regenerating fibres from the host brain were found to extend into this zone towards the graft.

9. While many of the residual nodules of the grafted ganglia were found to be richly innervated by outgrowths of fibres from the surviving ganglion cells, they appeared to exert no influence on the fibres of the host brain.

10. Emphasis is laid on the need to distinguish traumatized fibres in the host brain, which have become dislocated and distorted by the reaction of the host tissues to the graft, from true regenerating fibres.

11. Regenerating fibres from surviving ganglion cells may extend some way along blood vessels which enter the graft from the host tissues, but no evidence was found of regenerating fibres from the host brain extending along these vessels towards the graft.

12. It is concluded that if the intrinsic fibres of the host brain have any regenerative capacity, it is relatively feeble in comparison with the regenerative capacity of peripheral nerve fibres.

The completion of the work on which this report is based has been made possible by the expert co-operation of Mr E. Thompson and Mr W. Chesterman of this department. The former was responsible for the preparation of the extensive series of serial sections, and the latter for the photomicrography.

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EXPLANATION OF PLATES 1–3

Plate 1

Fig. 1. Low-power view of the grafted nerve fragment in Exp. 7. The graft reaches the surface of the cortex above, and below extends across the ventricular cavity to become adherent to the hippocampus. Note the absence of a definite layer of fibrous tissue separating the hippocampus from the adjacent portion of the graft. Pyridine-silver preparation. ×26.

Fig. 2. Normal spinal ganglion cell, stained with methylene blue. Note the satellite cells (of which nine can be seen in the section) lining the inner surface of the mesothelial capsule. ×300.

Fig. 3. Surviving cells of the grafted ganglion in Exp. 1 (section 29·5). Below, regenerating processes of the ganglion cells extend into the grafted tissue. Above can be seen the fibre plexus of the host cortex. Between the latter and the ganglion cells is a zone of relatively loose tissue made up of small round cells. None of the cortical fibres extend into this zone towards the graft. Pyridine-silver preparation. ×350.

Fig. 4. Surviving cells of the grafted ganglion in Exp. 1 (section 26·8). Regenerating fibres from the cells form a plexus of interweaving and dichotomizing fibres in the cellular masses formed by the proliferation of satellite cells. Below is seen a paraphytic process of one of the ganglion cells, ending in bulbous enlargements. Pyridine-silver preparation. ×350.

Fig. 5. Section through the zone of rounded cells separating the host cortex from the grafted ganglion in Exp. 1 (section 24·5). Below is seen the fibre plexus of the host cortex, and extending from this into the cellular infiltration is a relatively long fibre which shows irregular beading. Towards its termination it becomes sharply bent in a recurrent direction. This fibre appears at first sight to be in the process of regeneration, but it is probably a traumatized pre-existing fibre of the host cortex which has been distorted by the cellular infiltration. Near its proximal end is another cortical fibre ending in an oval bulb. Pyridine-silver preparation (retouched). ×700.

Fig. 6. Surviving ganglion cells in the grafted ganglion of Exp. 1 (section 27·12). In relation to the axone of the lowest cell is a rich periglomerular plexus. Several deeply impregnated bulbous expansions of newly formed cell processes are also to be seen. Pyridine-silver preparation. ×350.

Fig. 7. Regenerating fibres within an endoneural sheath of the grafted ganglion in Exp. 1 (section 24·2). The fibres all occupy a single sheath, to the inner aspect of which they are closely applied. From one of the fibres (to the right) a collateral branch extends transversely round the inner aspect of the sheath. Pyridine-silver preparation. ×850.

Fig. 8. Two ganglion cells in the grafted ganglion of Exp. 1 (section 29·13). These cells are grossly deformed, showing a coarse lobulation (*en fausse bipartition*). Pyridine-silver preparation. ×540.

Fig. 9. The termination of the fasciculus of regenerating fibres shown in Fig. 7 (section 23·13). Pyridine-silver preparation. ×850.

PLATE 2

Fig. 10. Pericellular plexuses formed round surviving ganglion cells in Exp. 1 (section 28·12). The ganglion cells themselves are out of focus. In the centre, a regenerated process is seen to have spun itself into a closely woven spiral. Above and to the right are some darkly impregnated bulbs formed at the extremities of paraphytic outgrowths. Pyridine-silver preparation. ×700.

Fig. 11. A branching fibre extending from the white matter in the direction of the grafted nerve fragment in Exp. 7 (section 40·12). The position of the graft is to the right, and is not included in the photomicrograph. The beaded and branching appearance suggests active regeneration; in fact, however, it is probably the traumatized remains of a pre-existing fibre. Pyridine-silver preparation (retouched). ×360.

Fig. 12. Section of the grafted nerve fragment in Exp. 7 (section 35·6). Two regenerating fibres are seen growing along an endoneural sheath. In the centre, they skirt the margins of a macrophage element. A number of Schwann bands are evident. Pyridine-silver preparation. ×550.

Fig. 13. Showing the margin of the grafted nerve fragment in Exp. 7 (section 35·1). To the left is the fibrous capsule covering the grafted tissue. To the right are seen fibres of the white matter of the host brain. Extending from the white matter is a circumscribed bundle of fibres which gives the appearance of having grown out towards the graft. Probably, however, they are pre-existing fibres which have become secondarily displaced by the cellular infiltration around the grafted tissue. Pyridine-silver preparation. ×400.

Fig. 14. Showing a residual nodule innervated by newly regenerated fibres in Exp. 7 (section 39·11). Four fibres extend along a strand of Schwann cells towards the nodule, and of these one is seen in the photograph to turn up, away from the main strand, to follow another cellular element. Within the nodule the fibres spin a close plexus. Pyridine-silver preparation. ×550.

Fig. 15. Closely packed fasciculi of nerve fibres running in close relation to a blood vessel situated in granulation tissue, from Exp. 7 (section 24·3). If these fibres are followed section by section, they are found to penetrate the fibrous sheath covering the implanted nerve fragment, and to gain the endoneural sheaths within the latter (see fig. 20). Pyridine-silver preparation. ×730.

Fig. 16. Showing the zone of round-celled infiltration separating the grafted ganglion from the cortex of the host brain in Exp. 1 (section 27·13). Above is seen the fibre plexus of the host cortex. In the infiltration zone are seen two fibres which have grown out from the implanted ganglion cells towards the cortex. Both end in globular expansions. Note that there is no evidence of any regeneration of the fibres of the cortex towards the graft. Pyridine-silver preparation (retouched). ×350.

Fig. 17. A neurotized residual nodule from Exp. 1 (section 28·2). Two regenerated nerve fibres are seen entering the nodule to ramify therein in a plexiform manner. Pyridine-silver preparation. ×700.

Fig. 18. Pericellular plexus around a ganglion cell in the grafted ganglion of Exp. 1 (section 27·2). Pyridine-silver preparation. ×540.

Fig. 19. The margin of the grafted ganglion in Exp. 7 (section 41·2). Several surviving ganglion cells are seen. To the right is the cortex of the host brain in which can be seen numerous fine fibres. Regenerated processes of the ganglion cells have penetrated into the thin capsule which separates the ganglion from the cortex, but they do not actually reach the latter. In the centre of the photomicrograph, one of these processes ends in a swollen terminal. Pyridine-silver preparation. ×300.

Fig. 20. A regenerating fibre entering the grafted nerve fragment in Exp. 7 (section 27·11). To the left is a relatively dense layer of fibrous tissue covering the graft, and to the right are the endoneural tubes of the latter. The fibre has penetrated the capsule and is entering one of the endoneural tubes. Pyridine-silver preparation (retouched). ×540.

Fig. 21. The margin of the grafted nerve fragment in Exp. 7 (section 31·8). The arrow points to a regenerated fibre which extends for some distance within the grafted nerve. Above are seen fibres in the layer of white matter covering the hippocampus, to which the graft is adherent. Note that there is no layer of fibrous tissue separating the graft from the white matter of the host brain; yet the fibres of the white matter show no tendency to enter the graft. Pyridine-silver preparation. ×550.

PLATE 3

Fig. 22. Fibres of the white matter of the host brain orientated towards the graft in Exp. 13 (section 70·3). The graft is to the left of the field shown in the photomicrograph. The fibres appear to be penetrating into a secondary capsule which has been formed in the tissues of the host brain around the graft. To the right (just above the centre) is a fasciculus of fibres which is bent back upon itself. Protargol preparation (retouched). ×400.

Fig. 23. Fibres of the white matter of the host brain orientated towards the graft in Exp. 13 (section 68·1). The graft is to the left of the field shown in the photomicrograph. To the right can be seen the feltwork of fibres in the white matter. From this a number of closely packed fasciculi appear to penetrate into a secondary capsule which has been formed in the tissues of the host brain around the graft. Some of the fibres of these fasciculi turn at right angles to extend along the cells forming the capsule. Protargol preparation (retouched). ×300.

Fig. 24. A regenerating fibre within the grafted nerve fragment in Exp. 9 (section 12·6). Protargol preparation. ×660.

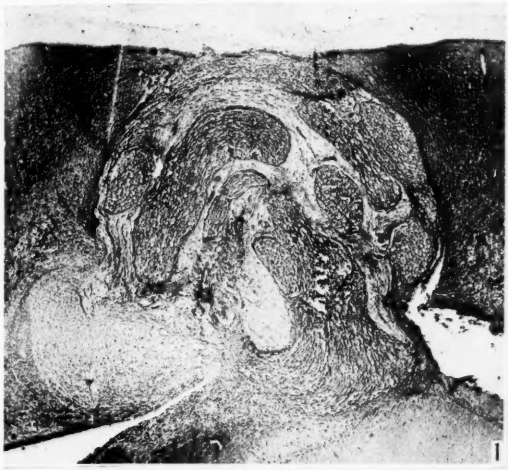
Fig. 25. Proliferating mass of Schwann cells at the tip of the grafted fragment in Exp. 13 (section 70·3). Protargol preparation. ×270.

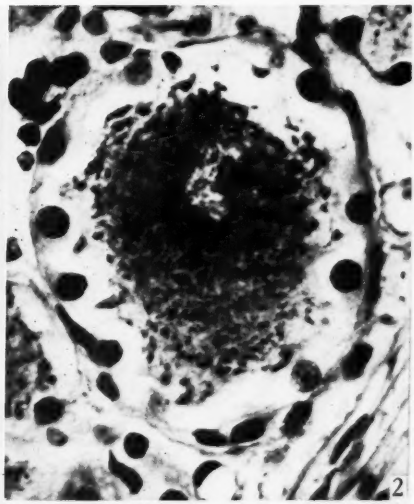
Fig. 26. Plexiform arrangement of regenerating fibres in relation to infiltrating Schwann elements in Exp. 13 (section 70·2). Protargol preparation. ×400.

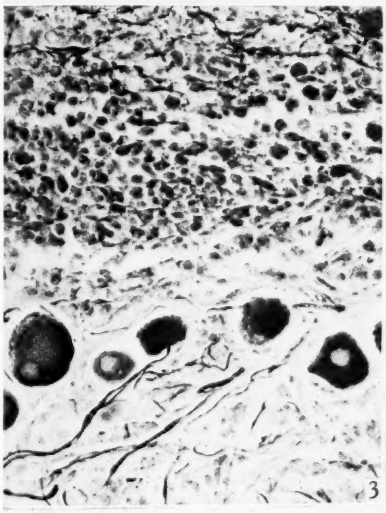
Fig. 27. The margin of the grafted ganglion in Exp. 1 (section 30·2). Below is the tissue of the ganglion containing fibres which have regenerated from the surviving ganglion cells. Above is the rich fibre plexus of the cortex of the host brain. Extending across the zone of round cells separating the two is a newly formed blood vessel which runs from the cortex to vascularize the graft. Although in some sections fibres from the ganglion cells extend a little way along this vessel towards the cortex, no cortical fibres extend in the reverse direction. Pyridine-silver preparation. ×300.

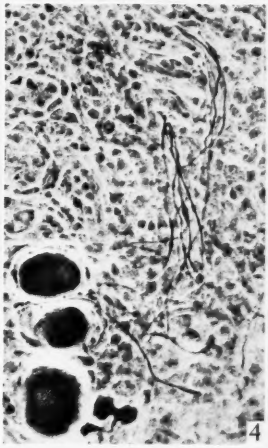
Fig. 28. Schwann bands in the grafted nerve fragment of Exp. 7 (section 34·9). Among the Schwann elements can be seen numerous macrophages. Pyridine-silver preparation. ×350.

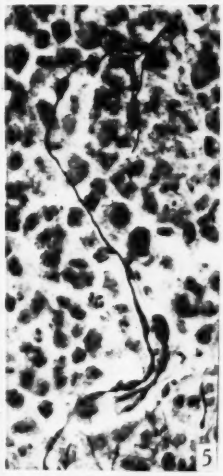
Fig. 29. A leash of regenerating fibres associated with a strand of invading Schwann cells in Exp. 13 (section 70·4). Above, one of the fibres can be seen dichotomizing. Below the centre is a nerve fibre which has turned back on a recurrent course. Protargol preparation. ×400.

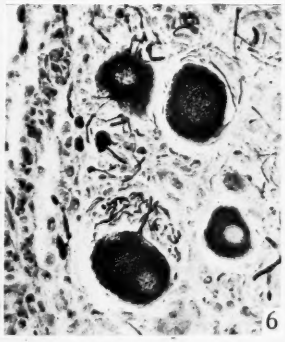


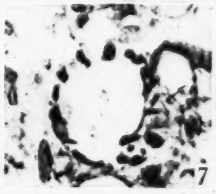


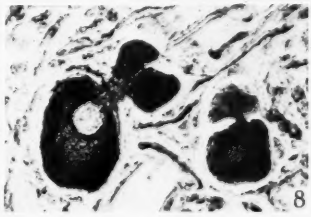












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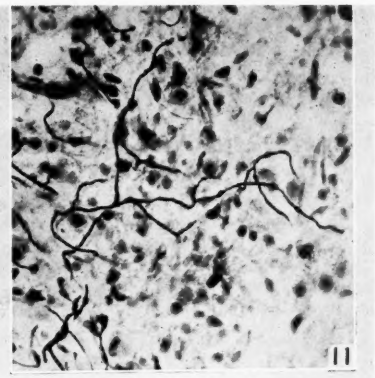
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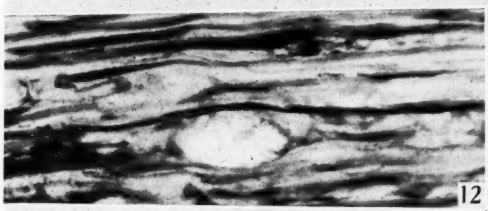
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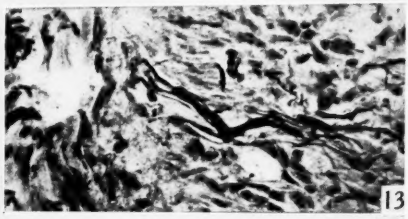
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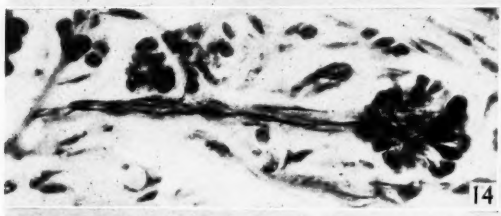
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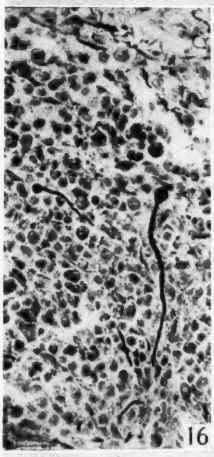
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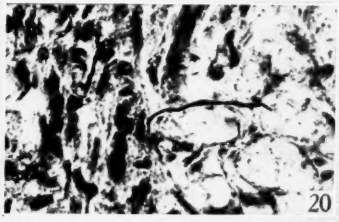
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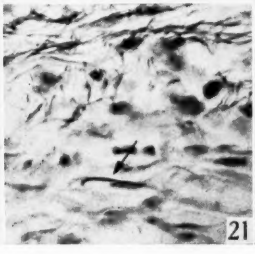
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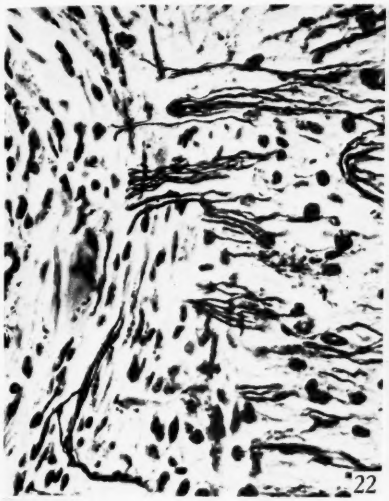
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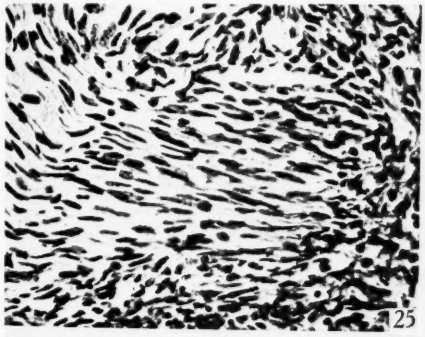
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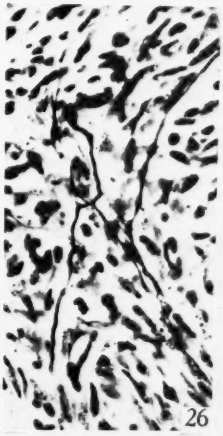
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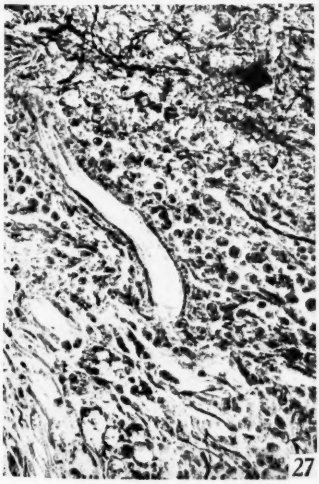
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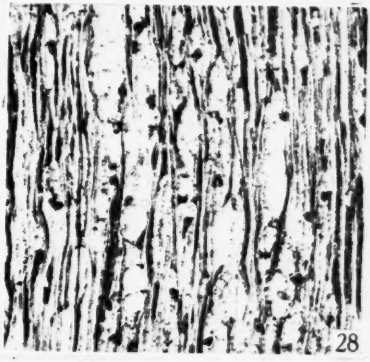
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UNIV OF MICH.

AXONAL REGENERATION IN CUTANEOUS NERVE PLEXUSES

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In a recent paper (Weddell & Glees, 1941) a description was given of the early stages in the degeneration of cutaneous nerve fibres, based upon the study of large whole preparations of skin in which the nerve fibres and their endings had been stained intravitally with methylene blue. It was pointed out that the method of vital staining with weak solutions of methylene blue, followed by the examination of whole preparations, has two outstanding advantages; it permits the course of individual nerve fibres to be followed in continuity for long distances, and, since the finest nerve fibres are stained with constancy only by methylene blue, it provides a more precise picture of the more delicate histological changes which occur during the course of degeneration than can be obtained by the more commonly employed silver methods. It was also observed that complicating factors which tend to obscure the behaviour of degenerating axones and their sheaths in nerve trunks, such as the concomitant changes in vascular tissues and the presence of large numbers of macrophages, are practically absent during the degeneration of the fine nerve bundles of the cutaneous plexuses.

In the present investigation an attempt has been made to analyse the pattern of regeneration of cutaneous nerve fibres by a similar method. The mode of application of the methylene-blue technique is believed to justify the reinvestigation of certain aspects of nerve regeneration, even though a vast amount of work on this problem has already been completed (the number of publications on nerve regeneration alone collected by Rossi & Gestaldi up to 1934 amounted to 928). Further, although many excellent accounts have been given of nerve regeneration (Howell & Huber, 1892; Marinesco, 1906; Perroncito, 1907; Poscharissky, 1907; Dustin, 1910; Ranson, 1912; Kirk & Lewis, 1917; Berblinger, 1918; Cajal, 1928; Nageotte, 1932; Boeke, 1935), in the majority the investigations have been confined to the changes which occur in nerve trunks, and the observations have been based upon the examination of thin sections and not of whole preparations. There have also been relatively few observations on the regeneration of cutaneous nerve fibres. Boeke & Heringa (1924), Dijkstra (1933), and Jalowy (1935), have examined skin from specialized areas in man, birds and monkeys, but here again the observations were based upon thin sections of silver-impregnated material which do not permit of the study of the pattern of nerve regeneration over large areas of denervated skin.

MATERIAL AND METHODS

Skin from the dorsum of the ears of albino rabbits was used. It was removed at intervals ranging from 2 days to 12 months after crushing of the main dorsal ear nerve at the base of one ear in a series of twenty rabbits. In nine animals the nerves were divided and gaps of 2 mm. or more left between the ends; skin from the dorsum of the ears in these animals was removed at intervals ranging from 2 weeks to 18 months. In four rabbits, in addition to crushing the main dorsal ear nerve, an attempt was

made to denervate the back of the ear completely by crushing the nerve trunks which pass up the medial margin of the ear.

Sensory tests were applied by pin pricks and by faradic stimuli from a 'Palmer' induction coil through fine stigmatic bipolar electrodes of interpolar distance 1 mm. The tests were carried out in each case just previous to the removal of skin from the ear, and in a number of cases also during the course of regeneration. The method of carrying out such tests has already been described in detail in a previous publication (Weddell, Guttmann & Gutman, 1941). Briefly, the stimulus is applied to the skin at intervals of approximately 1–1½ sec., working from the area of sensory loss towards the innervated area until a withdrawal response is obtained. This spot is then marked on the skin with Indian ink. At the end of the test the series of spots so obtained are tattooed into the skin. Since the skin of the ear is thin and the ventral surface of the ear remains innervated throughout the experiment, a finely adjusted faradic stimulus was found to be more accurate for routine use than a pin prick; the results obtained by either method were found to be strictly comparable provided approximately threshold stimuli (determined on the opposite normal ear) were used.

A 0·02 % solution of B.D.H. standard methylene-blue stain in 0·9 % sodium chloride A.R. containing 1 % procaine hydrochloride was used, the time allowed for the staining after injection being ¾ hr. Further details of the method have been given previously (Weddell & Glees, 1941).

OBSERVATIONS

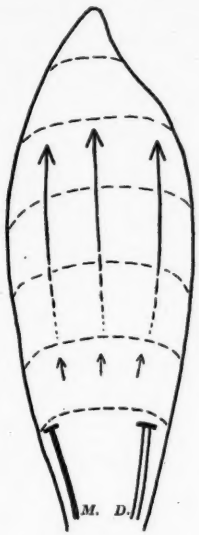
Sensory tests

(*a*) *After nerve crushing.* Where the main dorsal ear nerve only has been crushed the area of sensory loss usually involves the lateral (posterior) three-quarters of the dorsum of the ear, but the actual size and shape of the desensitized area are very variable. As early as the second day after operation there is a slight diminution in the area of sensory loss, the demarcation line having moved a small distance distally and laterally towards the denervated zone; thereafter the area of sensory loss slowly diminishes until at the end of 14 days the advancing margin of sensibility has reached some 6–7 mm. beyond the original line of demarcation. After this time the rate of advance increases considerably, reaching an average speed of 2–2·5 mm. a day. The area of sensory loss continues to diminish in a distal and lateral direction (Text-fig. 1*b*).

Where the dorsum of the ear has been completely denervated by nerve crushing (and only two cases proved to have been so denervated), the regeneration does not commence until 14 days have elapsed and then the advancing margin of sensibility progresses at a rate of about 1 mm. a day. The line of demarcation is difficult to define in these cases, but, ignoring certain irregularities related to precocious growth along main nerve trunks, there is a fairly straight transverse line of returning *cutaneous* sensibility, advancing up the ear, the last part of the ear to become re-innervated being the tip (Text-fig. 1*a*).

(*b*) *After nerve section.* Where only the main dorsal ear nerve has been cut and a gap of 2 mm. or more left between the ends, sensory tests carried out 2, 3 and 4 weeks following the operation show that there is a steady increase in size of the area of skin from which nociceptive responses can be elicited. The margin demarcating the sensitive from the insensitive areas advances approximately 0·5 mm. a day. As will be seen

later, this is the rate of growth of nerve fibres extending from surrounding normal nerves towards the denervated area.



Text-fig. 1*a*.

Text-fig. 1*b*.

Text-fig. 1*a*. Diagram illustrating the progress of sensory recovery after section of all the nerves passing up to the dorsum of the rabbit's ear. *D.*, main dorsal ear nerve; *M.*, nerves passing up the medial (anterior) margin of the ear.

Text-fig. 1*b*. Diagram illustrating the progress of sensory recovery after section of the main dorsal ear nerve only. *D.*, main dorsal ear nerve; *M.*, nerves passing up the medial (anterior) margin of the ear.

General pattern of regeneration

(*a*) *After nerve crushing.* By the exclusive use of albino rabbits, and by taking care that the methylene blue is evenly injected throughout the skin of the dorsum of the ear, it is possible to observe the progress of regeneration with a hand lens or even macroscopically. In the cleared specimens all the nerves, and to a lesser degree the blood vessels, are stained blue, while the rest of the tissue remains colourless. The blood vessels which do take up the stain can be readily distinguished from nerve trunks (Pl. 1, fig. 1). The pattern assumed by the advancing fibres is well seen 4 weeks after crushing the main dorsal ear nerve only. By this time all the main nerve trunks in the originally denervated area contain regenerated fibres and therefore take up the stain. These fibres have not yet extended into the small cutaneous nerve bundles, with the exception of a narrow zone alongside the normal intact nerve bundles passing up the medial margin of the ear. The reason for the precocious innervation of this zone is not clear and is further discussed below.

Five weeks after crushing the main dorsal ear nerve, the only portion of skin in which no small nerve bundles were seen was an area situated at the lateral margin of the ear three-quarters of the way towards the tip. This area was insensitive to nociceptive stimulation.

A study of ears at earlier stages of regeneration confirms the impression that regeneration is actually more rapid along the original main nerve trunks than in the cutaneous bundles, and that in the main nerve trunks it is also more rapid where the latter lie in closest proximity to normal intact nerves.

After complete denervation of the dorsum of the ear, the last area of skin to become re-innervated is in the midline towards the tip, nerve fibres passing first along the original main nerve trunks and only later along the smaller nerve bundles. It is interesting to note that the density of re-innervation in the latter always appears to be greater in the neighbourhood of the larger blood vessels, even when the vessels lie at some distance from the main nerve trunks (Pl. 1, fig. 1). It is possible therefore that the rapidity of regeneration of the cutaneous nerve bundles in any particular area of skin is related to its vascularity.

(*b*) *After nerve section.* After the main dorsal cutaneous nerve had been cut and a gap of 2 mm. or more left between the ends, it was found that even 4 weeks after operation practically no regeneration had taken place along the distal stump. In this case, however, definite histological evidence was obtained that new sprouts had grown out from normal intact nerve fibres at the medial margin of the ear and had extended for a short distance into the denervated zone.

Correlation of sensory tests with the pattern of regeneration

On comparing sensory tests with the general pattern of regeneration it was found that the margin of regeneration as indicated by the methylene-blue staining was always about 5 mm. in advance of the area from which nociceptive responses could be elicited. The reason for this discrepancy is discussed below. Faradic stimuli confirmed the histological evidence that nerves advance quicker along the nerve trunks than along the intervening cutaneous nerve plexuses.

In the experiment in which, after section of the main dorsal ear nerve, histological evidence was obtained that fibres had sprouted from adjoining normal nerves, there was a remarkably close correlation between the area from which nociceptive sensations can be aroused and the newly innervated area.

Histological observations

At the site of lesion. Two days after crushing the main dorsal ear nerve, regenerating nerve sprouts have penetrated into the cellular exudate between the nerve stumps; the necrotic segments of axones of the proximal stumps are still evident. Terminal swellings are seen at the end of many regenerating axones and their collaterals. Direct and proximal sprouting can be seen, and emerging from the terminal clubs are brushes of fine fibres which appear similar to those described by Cajal (1928) as direct sprouting by 'ravelling'. Net formations (Cajal, 1928) are also seen. With the exception that there is more multiple sprouting than is usually described after nerve section the picture is essentially similar to that given by Poscharissky (1907), Kirk & Lewis (1917), Cajal (1928), Boeke (1935) and other workers. There is, however, one feature of these methylene-blue preparations which deserves emphasis. A comparison of the descriptions and illustrations of other authors makes it probable that the finest fibres which can be demonstrated by intravital staining with methylene blue are often not brought to view by silver impregnation methods. Hence the picture of regenerating nerve fibres in methylene-blue preparations appears somewhat more complicated than might

be expected from previous descriptions. Even with the use of methylene blue, however, the actual terminations of the finest sprouts cannot be defined with certainty, for in some cases they are not surmounted by growth cones; on the contrary, they become progressively finer in diameter and finally pass beyond the powers of resolution of a microscope with a 1/12 in. oil immersion lens.

In the peripheral stump. The rate of regeneration of individual fibres along the peripheral stump is very variable; for some fibres 'get through' without any apparent hindrance. In one case two fibres were found to have advanced 40 mm. along the trunk 8 days after crushing the main dorsal ear nerve. The majority of such rapidly regenerating fibres are extremely fine and, unless the growing tip is expanded to some extent, their precise termination is impossible to define with certainty. Most fibres regenerate much more slowly and, as will be seen later, the more advanced fibres do not necessarily become mature or functional.

One of the most striking features in the proximal part of the distal stump, 2 weeks after crushing the main dorsal ear nerve, is the large number of fine newly regenerated fibres undergoing degenerative changes as compared with the number seen more distally. In many cases, these fine degenerating fibres can be traced back to their origin as sprouts from proximal stumps. The great majority of regenerating axones have smooth homogeneous outlines though they are not of uniform diameter. Throughout the course of the fibre there are fusiform swellings which usually occur at fairly regular intervals (Pl. 1, fig. 2).

In the smaller nerve trunks and cutaneous nerve plexus. The finest visible terminations of the nerve fibres are associated with Schwann cell pathways which retain the pattern of the cutaneous plexus in the normal ear (Pl. 1, fig. 5). On no occasion in the rabbit's ear has the tip of a regenerating nerve fibre in the cutaneous plexus been seen pursuing a course independently of a Schwann band. This is made clear by the fact that, for about 1 mm. ahead of the finest visible sprouts of nerve fibre which are detectable under the oil immersion, the nuclei and the cytoplasm of the Schwann bands are commonly stained in a characteristic fashion by intravital methylene blue (Pl. 1, fig. 4). The Schwann bands are, however, only stained when regenerating nerve fibres are growing along their course and only in relation to regenerating nerve fibres of fine diameter, for when traced proximally the bands become progressively less stained until they are no longer visible. In no case have mitotic figures been seen in Schwann band nuclei of the cutaneous nerve plexus which are stained with methylene blue. It may be noted, also, that it has not been possible with this technique to see cell boundaries between individual nuclei along the course of a single Schwann band.

In order to illustrate the mode of regeneration of nerve fibres through the cutaneous nerve plexus, and to demonstrate their relationship to stained Schwann bands, a series of photomicrographs was taken of a nerve fibre in its course from a small nerve trunk into the cutaneous plexus. Pl. 1, fig. 6, shows such a fibre dichotomizing where it enters the plexus. If one of the branches is followed the axis cylinder is seen to become visibly expanded at a point where the Schwann bands outlining the plexus separate. From this expanded terminal a large number of fine nerve fibrils proceed distally, apparently along the surface of the Schwann band (Pl. 2, fig. 7). Pl. 1, fig. 3, shows the appearance of such nerve fibrils somewhat further along their course, and Pl. 2, fig. 8, illustrates a point still more distally where the majority of the fibrils are so fine that they are approaching the limit of visibility. Just proximal to the expanded

terminal which gives rise to the fibrils the Schwann bands begin to stain with methylene blue and become progressively more darkly stained as the fibrils are traced distally. As already stated, they continue to take up the stain for a distance of not more than 1 mm. ahead of the finest visible nerve terminals.

With the technique employed (which involves alcohol fixation) it is difficult to say with certainty from a spread preparation whether the finest fibrils lie on the surface of the Schwann cytoplasm or whether they are situated just within it. Transverse sections of Schwann bands from selected areas at various stages of regeneration show that the larger nerve fibres are completely surrounded by Schwann cytoplasm but that the finest advancing fibrils lie towards the surface of the Schwann band and, in fact, in the most distal part of their course they appear to lie actually on the surface. Further evidence that the most distal extremities of the advancing fibres lie on the surface of the Schwann cytoplasm is furnished by some histological pictures in which one of the fine fibres may be seen to curve away from the Schwann band and rejoin it a little further on as though it had become detached during the course of preparation (Pl. 2, fig. 10).

Measurements made along twelve typical regenerating fibres showed that, although the most advanced nerve fibrils which are visible probably lie on the surface of the Schwann cytoplasm, at a distance of 3–4 mm. proximal to their tip they are completely surrounded by the cytoplasm. When, however, the distal extremity of a regenerating nerve fibre is surmounted by a growth cone (presumably indicating an obstruction to growth), the length of the part which is not surrounded by cytoplasm is considerably reduced and may even disappear (Pl. 2, figs. 9, 11).

Throughout the course of the regenerating fibres maturation is seen to be taking place. This involves the degeneration of a large number of collateral branches, and the degeneration seems primarily to affect those which had previously contained the largest number of fusiform swellings along their course, in other words, those which had presumably met with most obstruction during their outgrowth. As the number of fibres in the smaller nerve trunks and the cutaneous nerve plexus becomes reduced, the average diameter of the remaining fibres increases (Pl. 2, figs. 12–18). Concomitant with this increase in diameter is the disappearance of the fusiform swellings which form such a characteristic feature of nerve fibres in the early stages of regeneration (Pl. 2, figs. 9, 13, 21). This is well illustrated by comparing photomicrographs of two small nerve bundles taken 5 and 12 weeks respectively after crushing of the main dorsal ear nerve (Pl. 2, figs. 15, 20). On the contrary, it is also clear that the characteristic beading of the fibres of the subcutaneous and vascular nerve nets does not become apparent until maturation is completed.

In areas where regeneration is fairly advanced, fine nerve fibres can be seen approaching the skin where they begin to extend into a regenerating network distributed just beneath the epidermis. Owing to the staining of the Schwann cells at certain stages during regeneration, it is now possible to see that the Schwann cytoplasm accompanies the finest fibres through the greater part and perhaps the whole of their course (Pl. 3, figs. 22, 25). An observation of some interest is that the so-called 'Langerhans cells' are abundant in the skin of the albino rabbit's ears and these experiments show that, like the Schwann cells, they take up the blue stain when the fine regenerating nerve terminals are approaching them, i.e. when the terminals are just becoming apparent beneath the epidermis (Pl. 3, fig. 25). Boeke (1940) took the

view that these cells were in protoplasmic continuity with nerve terminals in the epidermis. Our preparations show no evidence of this; on the contrary, when they are stained with methylene blue they appear to lie in series with the Schwann cell elements which are related to the fibres in the subepidermal network. It thus appears not improbable that these 'cells of Langerhans' are nothing more than modified Schwann cells.

In tracing the course of the regenerating nerve fibres at intervals after experimental interruption, it soon becomes clear that those passing to blood vessels regenerate faster than those giving rise to nerve nets beneath the epidermis or ending around hair follicles. Fibres leave the nerve trunks and pass to the blood vessels along the course of persisting Schwann elements (Pl. 3, figs. 24, 26). During maturation, these vascular nerves, like those ending beneath the epidermis, at first appear to lie on the surface of the Schwann bands but later become surrounded by the Schwann cytoplasm. The cytoplasm related to a single Schwann nucleus extends along the individual components of the nerve nets on blood vessels for considerable distances (Pl. 3, figs. 24, 26). It is probable that these Schwann elements are identical with the 'interstitial cells' described by Lawrentjew (1926) and Boeke (1940), and thought by the latter to be nerve cells. Beading of these vascular nerves likewise does not occur until after maturation is completed (Pl. 3, fig. 23). The faster rate of regeneration of the nerve nets along blood vessels was determined by tracing the finest visible terminations along the line of the advancing cutaneous nerves, when in 15 counts along different vessels the vascular nerves were on the average 3–4 mm. ahead of the cutaneous nerves. It must be remembered, however, that nerve fibres advancing into the skin have to pass through a tortuous course in the cutaneous nerve plexus towards the skin surface, whereas vascular nerves advance more directly along the line of the vessel.

Sensory recovery

When, after nerve crushing, pin pricks first give rise to nociceptive reactions, it is found that the number of random pricks required to arouse a single such reaction is greater over a given area in which regeneration has just been completed than in a similar area in a normal ear. It is also found that a stronger faradic stimulus must be given in order to arouse nociceptive responses with each application of the electrodes. During recovery after nerve section a similar phenomenon is observed, except that nociceptive responses are still more difficult to obtain than in cases in which the nerve had simply been crushed; in addition, there is a definite latent period in the nociceptive reactions which follow the application of the stimulus. In the later stages of regeneration (3–12 months after nerve crushing) very little difference in the sensory recovery between the normal and operated side could be observed with the tests employed.

In areas of skin which have just reached a stage in which pin pricks and faradic stimuli arouse nociceptive responses, many hair follicles are supplied only by single mature nerve fibres of small diameter, while other fine fibres may be seen still in the process of advancing towards them along the Schwann bands. Fine nerve fibres can also be seen leaving the cutaneous nerve plexus and giving rise to nerve nets situated just beneath the epithelium. These nets, however, are isolated from one another and do not form a continuous interdigitating series as in a normal ear. Finally, in such areas of skin, there are always a number of stained Schwann bands along which nerve

fibres are advancing but which have, as yet, not reached their destinations; ahead of such advancing fibrils (which are always situated in the deeper portions of the cutaneous nerve plexus) the Schwann bands are stained for as much as 1 mm.

At a slightly later date a certain number of the fibres show the presence of nodes of Ranvier. No such nodes are visible until about 4 weeks after sensation (nociceptive) has returned (Pl. 2, figs. 19–21).

In the later stages of regeneration (3–12 months after crushing the main dorsal ear nerve) the only difference which can be detected in the pattern of cutaneous innervation as compared with a normal ear is an increase in the number of collateral fibres in the nerve trunks. In addition, the average diameter of the nerve fibres throughout the ear is somewhat smaller (Pl. 2, fig. 20). On the other hand, after nerve section, even when no sensory differences can be detected between the two sides, the following histological changes are seen. In the nerve trunk there are a great many collaterals and the fibres are in general of considerably smaller diameter than in a normal trunk. The hair follicles are all innervated by fibres which are much finer than usual, but the density of the subcutaneous nerve nets is normal. In addition, a very large number of fibres of the cutaneous nerve plexus are still surrounded by blue-stained Schwann cells. It is also clear, by tracing the nerve trunk fibres towards the point of section, that far fewer fibres penetrate into the distal stump than after crushing.

On comparing the results of sensory tests with the histological pattern of regeneration in the rabbit's ear, it is apparent that the finest growing tips of the nerve fibres, and even mature nerve fibres when they are few in number, are not sufficient to mediate impulses giving rise to nociceptive responses. In fact, the advancing line of sensory change lags behind the advancing nerve sprouts by at least 5 mm. It should be emphasized, however, that the finest advancing nerve sprouts are always situated at a deeper level beneath the skin, for they are here entering the cutaneous nerve plexus through which they must pass towards the skin surface. It may well be that the discrepancy between the sensory tests and histological observations is in part due to this fact, for in striking contrast to this is the correspondence between the two advancing boundary lines when nerve fibres are extending locally through the superficial layers of the plexus and subepidermal nets towards the area of sensory loss. In these cases they correspond to within 1 mm. This close correspondence is probably also related to the slow rate of progression of these new-growing nerve fibres, which thus undergo maturation almost as quickly as they advance. Further details of this local extension of nerve fibres have been given already (Weddell *et al.* 1941).

DISCUSSION

The histological observations on axonal regeneration in the present investigation are in general agreement with those made by the majority of workers, mentioned in the introduction, who studied thin sections of nerve trunks. The use of whole preparations has, however, enabled a certain number of new observations to be made; in addition, the process of nerve regeneration can be better seen as whole. For instance, it is clear that regenerating cutaneous nerve fibres in the smaller nerve trunks and cutaneous nerve plexus follow only the course of Schwann cell frameworks all of which persist apparently unchanged in pattern after nerve degeneration. This is important, for it suggests that in human skin regenerating nerve fibres must approach the connective tissue elements of specific end-organs which also are said (Boeke, 1940) to retain their

position in the skin following nerve degeneration. Whether they approach their *correct* end-organs, however, remains uncertain.

It has been shown that typical regenerating nerve fibres in the cutaneous nerve plexuses are not surmounted by growth cones but that the termination of such fibres is so fine as to pass beyond the powers of resolution of the microscope. It has also been shown that Schwann bands are stained for 1 mm. ahead of these fine nerve terminals; it is interesting to note that staining of the Schwann bands in advance of regenerating nerve fibres has also been described in nerve trunks by Poscharissky (1907) using Stroebe's osmic acid or aniline blue staining after fixation in Flemming's fluid.

The rate of regeneration of the numerous collateral sprouts along the surface of the Schwann cells in the nerve trunks is variable and only a few of these sprouts mature and become surrounded by the Schwann cytoplasm. It is thus almost certain that in any transverse section of a regenerating nerve trunk some fibres will appear to be lying on the surface of the cytoplasm and others within it. In fact, an illustration from Cajal (1928, Fig. 81, p. 199) shows such an arrangement, although this is not specifically referred to by the author, and photomicrographs illustrating the same appearance have been given by Kirk & Lewis (1917). Whether the finest visible nerve sprouts are really external to the Schwann cytoplasm or whether they lie just within its surface layer is difficult to determine by any histological method on fixed tissues; it has been noticed, however, that the fibres do appear to be fairly easily detached from the cytoplasm without destruction of the latter, and it is thus inferred that they are external to it (Pl. 2, fig. 10). Ranson (1912), Cajal (1928) and Boeke (1935) believed that regenerating fibres lie within the cytoplasm of the Schwann cells, while Perroncito (1907) believed that they are always on the surface. Poscharissky (1907) and Kirk & Lewis (1917) showed that regenerating fibres may be either within or without the Schwann cytoplasm. These authors also described degenerative changes in the fibres located at the surface. It is evident that these conflicting statements are related to the fact that there is a change in relationship between the Schwann cytoplasm and axis cylinders during the course of maturation. The factors responsible for maturation can only be conjectured, but it seems that those fibres which become surrounded by Schwann cytoplasm continue to mature and those which for some reason do not gain the protection of the Schwann cytoplasm degenerate.

It has been shown in this investigation that all the Schwann bands outlining the position of the cutaneous nerve plexus in the later stages of regeneration are associated with fine nerve fibres even though by no means all the fibres in the proximal stump have sent out sprouts which have crossed the scar. Thus at least some of the Schwann bands must have become associated with collaterals. It also appears likely, since each Schwann band has a fibre related to it, that once maturation is completed no more fibres can cross the scar on to the surface of the bands with the possibility of survival. It follows from these observations that the extent of recovery of sensory innervation in the skin from the histological point of view does not depend mainly upon the number of fibres in any given nerve trunk supplying the skin but upon how many of the fibres are collaterals derived, more proximally, from a single source. A comparison of the average diameters of the individual fibres in normal and regenerated nerve trunks gives some histological evidence of the extent of sensory recovery, for the greater the number of collaterals the smaller is the average diameter of each nerve fibre. According to Ranson (1912) a single regenerating nerve fibre from the proximal

stump of a divided nerve may give rise to as many as fifty new sprouts. This estimate has been confirmed in the present investigation. Potentially, therefore, this greatly increases the chance of nerve fibres 'getting through', but as explained above the penetration of fibres into the distal stump does not mean that there will be a corresponding return of sensation of normal quality. It indicates only that there is a high probability of complete *histological* re-innervation of the distal stump and the skin, but the re-innervation may be effected by many fibres of reduced diameter. It has already been shown that the finest fibres do not themselves give rise to impulses which produce nociceptive responses when the usual stimuli are applied. It thus becomes clearer why the physiological quality of regeneration after crushing a nerve is so much better in the earlier stages than after nerve section. It is true that no difference in the general response to nociceptive stimulation in the later stages of regeneration after nerve section is observed, but the stimuli used are hardly adequate to detect finer changes in the quality of sensation.

In a previous publication (Weddell & Glees, 1941) a peculiar staining reaction was noticed in the intermediate zones (zones of overlap between normal and degenerating nerve fibres) following methylene-blue injection. In certain parts of these zones the Schwann bands become stained and the cytoplasm surrounding degenerating myelin globules takes on a fibrillary appearance. Illustrations of these processes were given and it was suggested that they most closely resembled local attempts at regeneration. It is now clear that these appearances are due to a local extension of undamaged nerve fibres both within the intermediate zone and towards the denervated area (Weddell *et al.* 1941). This process takes place in the same manner as regeneration from the proximal stump of a divided nerve, but is confined to fine nerve fibres of the cutaneous plexus.

The observation that Schwann cytoplasm accompanies the finest fibres of the subepidermal nerve net through the greater part and perhaps the whole of their course, and that the so-called 'Langerhans cells' are probably modified Schwann cells, is in accord with Trotter's interesting generalizations on the insulation of the nervous system (Trotter, 1924). Indeed, it appears that these generalizations may be extended to include what he, in common with most authors, describes as naked nerve terminals, intra-epidermal nerve fibres and nerve nets surrounding blood vessels, for these fibres seem also to be surrounded by delicate prolongations of Schwann cytoplasm as far as their terminations.

In two recent publications (Weddell, 1941 *b*, *c*) it has been suggested that since cutaneous sensory spots are each innervated by multiple nerve fibres, during the course of regeneration these will arrive at each separate spot at different times because they approach it from different directions and the ultimate course followed by the individual fibres will necessarily be of different lengths. Thus there will be a phase during regeneration in which each sensory spot is innervated by a single instead of by multiple fibres. Such a stage in regeneration has now been found to occur and hence the physiological findings of Trotter & Davies (1909) and Boring (1916) are seen to have an anatomical basis as far as the process of regeneration of cutaneous nerve fibres in the ear of the rabbit can be applied to that in man. The more rapid regeneration of nerve fibres along blood vessels is also in accord with the observations of Trotter & Davies (1909), who observed that vasomotor control returned more rapidly than skin sensation.

The general pattern of nerve regeneration throughout the skin of the whole ear has shown that regeneration of fibres from the injured nerve occurs first in the neighbourhood of adjacent normal nerves; this, it should be emphasized, is distinct from the extension of adjacent normal fibres which also occurs towards the area of sensory loss. The observation that cutaneous nerve fibres also regenerate more rapidly in the immediate neighbourhood of blood vessels suggests that both these occurrences may be in some way related to nutritional factors.

These phenomena evidently play some part in the process of diminution of areas of sensory loss which are observed clinically to take place from the periphery towards the centre of a degenerated area rather than from the centre outwards. An attempt was made to account for this phenomenon by relating it to the pattern in which the nerve fibres are distributed beneath the skin in specified areas (Weddell, 1941 *a*, *c*). In some cutaneous areas, however, the pattern of nerve distribution will not account for it entirely.

In contrast with this mode of innervation it can be seen clinically that the return of skin sensibility in regions not surrounded by areas innervated with normal nerves, such as the digits, is by an advancing wave of sensation along the line of the cutaneous nerves supplying the skin.

The more rapid regeneration of cutaneous nerve fibres in the neighbourhood of blood vessels may serve to explain the bridges of cutaneous sensibility which are commonly found, clinically, to cross an area of sensory loss during the course of regeneration from the proximal stump of the divided nerve.

It has been seen that the process of nerve regeneration throughout an area of skin involves first the rapid regeneration of nerve fibres along the main subcutaneous nerve trunks, and that it is only at a later date that fibres extend from these trunks towards the skin by way of cutaneous nerve plexuses. In other words, regenerating nerve fibres ramify in the subcutaneous tissues some time before they give extensions to the skin itself. This is an important fact from the point of view of the nociceptive sensory tests which are used to determine the progress of sensory recovery. For instance, it has been found in clinical cases that 'deep pricks' (2–3 mm. deep) will arouse pain in an area which is still anaesthetic to lighter pricks ($\frac{1}{2}$–1 mm. deep). This furnishes an explanation for the statement that one of the earliest indications of recovery of skin sensibility is a painful sensation caused by pinching the skin, for this manipulation necessarily involves the subcutaneous tissues. In any case the importance of using standard instruments, which will give approximately constant stimuli from day to day in the same patient, cannot be over-emphasized, if a true picture of the course of sensory recovery (nociceptive) is to be obtained. Cobb (1919) has already shown the importance of this from the clinical point of view.

SUMMARY

1. A descriptive account of the regeneration of cutaneous nerve fibres in the ear of the rabbit has been given.

2. The diminution of the areas of sensory loss following nerve interruption has been correlated with both the macroscopical and microscopical appearance of the regenerating nerve fibres.

3. Nerve fibres first advance along the original main nerve fasciculi beneath the skin. The first fasciculi to be re-innervated are those lying in closest proximity to

normal nerve trunks. It is also clear that cutaneous nerve bundles which are more closely related to the larger blood vessels are more rapidly re-innervated than those farther away.

4. When the nerve trunks have become re-innervated the nerve fibres pursue a tortuous course through the cutaneous plexus towards the skin surface. The finest visible terminations of the nerve fibres are associated with Schwann cell pathways which retain the pattern of the cutaneous plexus in the normal ear. On no occasion in the rabbit's ear has the tip of a regenerating nerve fibre in the cutaneous nerve plexus been seen pursuing a course independently of a Schwann band.

5. The Schwann bands become stained with intravital methylene blue during the course of regeneration when they are in relation with advancing nerve fibres of fine diameter. This staining first appears about 1 mm. in advance of the finest visible advancing nerve sprouts. No cell boundaries can be seen between the nuclei of a single Schwann band so stained, and no mitotic figures have been seen in Schwann bands of the cutaneous nerve plexus.

6. The growing tips of the finest nerve fibres cling to the surface of the Schwann cells; as they mature they become enclosed within the Schwann cytoplasm. This change in relationship is completed over a distance of 3–4 mm. in normally regenerating nerve fibres.

7. During the course of maturation the great majority of collaterals produced by a terminal growth cone degenerate and the remaining fibre or fibres increase in diameter.

8. The pattern of regeneration is discussed in relation to clinical findings and to the application of nociceptive stimuli for the determination of the position of a border of sensory loss.

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EXPLANATION OF PLATES 1–3

All photomicrographs reproduced on Plates 1–3 are from methylene-blue stained preparations of skin from the dorsum of the albino rabbit's ear.

PLATE 1

Fig. 1 (×5·5). Shows the pattern assumed by regenerating nerve fibres 4½ weeks after crushing the main dorsal ear nerve. The blood vessels can be readily distinguished from the re-innervated nerve trunks for the latter have a striated appearance. Nerve fibres can be seen entering the cutaneous nerve plexus in the neighbourhood of the nerve trunks and blood vessels. Owing to the low magnification extension into the cutaneous nerve plexus appears as a blurring around the nerve trunks and blood vessels, for the individual components of the plexus are too small to be recognized at this magnification. The areas between the nerve trunks in the upper part of the photograph are not yet re-innervated.

Fig. 2 (×550). Shows two regenerating nerve fibres in the peripheral stump of the main dorsal ear nerve which had been crushed 2 weeks previously. The fibre to the left gets progressively finer as it is traced distally until it passes beyond the powers of resolution of the microscope. The fibre to the right ends in a growth cone. A few granular remains of a degenerating collateral fibre can be seen towards the top at the right of the picture.

Fig. 3 (×900). Shows a Schwann band covered by a number of advancing nerve fibrils containing numerous swellings along their course. As the fibrils are traced distally, i.e. towards the lower end of the photomicrograph, they become progressively finer in diameter and the swellings become less obvious. The cytoplasm of Schwann bands very occasionally bifurcates forming two separate strands which soon reunite. Such a condition is shown in the upper part of this photomicrograph which was taken 5 weeks after crushing of the main dorsal ear nerve.

Fig. 4 (×900). Shows two nuclei in an apparently uninnervated Schwann band. The cytoplasm between the nuclei appears to be continuous. This Schwann band was in series with that shown in fig. 8, but is situated 1 mm. ahead of the finest visible advancing nerve fibrils. The photomicrograph was taken 5 weeks after crushing of the main dorsal ear nerve.

Fig. 5 (×350). Shows branches from a regenerating nerve fibre passing along persisting Schwann elements of the cutaneous nerve plexus. As the fibres are traced distally the nuclei and cytoplasm of the Schwann bands become more darkly stained. The photomicrograph was taken 5 weeks after crushing of the main dorsal ear nerve.

Fig. 6 (×825). Shows a medium-sized nerve fibre (equivalent in diameter to a finely myelinated fibre in a normal rabbit's ear) about to enter the cutaneous nerve plexus where it undergoes repeated dichotomization. The photomicrograph was taken 5 weeks after crushing of the main dorsal ear nerve.

PLATE 2

Fig. 7 (×675). Shows a stained Schwann band covered by a large number of nerve fibrils sprouting from the swollen end of an axis cylinder which can be seen in the top left-hand corner of the picture. The nerve trunk giving rise to these fibrils was a continuation of one of the branches of the nerve fibre shown in fig. 6. The photomicrograph was taken 5 weeks after crushing of the main dorsal ear nerve.

Fig. 8 (×1000). Shows a stained Schwann band covered by a large number of very fine nerve fibrils. Just below the upper Schwann nucleus there is a small end-bulb formed by a fine advancing fibre which can be seen in the upper part of the picture. Advancing from the tip of this end-bulb are a number of fibrils. When this band was traced distally the nerve fibrils became less obvious until the condition shown in fig. 4 was reached. The photomicrograph was taken 5 weeks after crushing of the main dorsal ear nerve.

Fig. 9 (×575). Shows a regenerating nerve fibre which has become arrested. The cause of the arrest was not clear but the advancing tip was surrounded by dense fibrous tissue. The fibre is of much greater diameter than surrounding fibres which have not become arrested. No stained Schwann cells surround the fibre and no collaterals are seen in relation to it. The photomicrograph was taken 4 weeks after crushing of the main dorsal ear nerve.

Fig. 10 (×800). Shows a fine advancing nerve fibril curving away from a Schwann band and rejoining it a short distance further on. The photomicrograph was taken 5 weeks after the main dorsal ear nerve was crushed.

Fig. 11 (×1075). Shows an extremely fine nerve fibre surmounted by a growth cone. This fibre has probably become ensheathed within Schwann cytoplasm, for the Schwann elements are no longer stained. The fine fibrils which leave the cone become associated with stained Schwann elements in the more distal part of their course. This photomicrograph was taken 5 weeks after crushing of the main dorsal ear nerve.

Fig. 12 (×600). Shows a stage in the process of maturation. The collateral which contains the large fusiform swellings was found to be disintegrating when traced more distally. The photomicrograph was taken 5 weeks after crushing of the main dorsal ear nerve.

Fig. 13 (×800). Shows a disintegrating collateral lying beside a normal fibre which contains a number of swellings along its course. The photomicrograph was taken 5 weeks after crushing of the main dorsal ear nerve.

Fig. 14 (×550). Shows the point of bifurcation of a nerve fibre. One branch is degenerating and was found to have disintegrated completely when traced more distally, while the other appears to be increasing in diameter. The photomicrograph was taken 5 weeks after crushing of the main dorsal ear nerve.

Fig. 15 (×600). Shows a small nerve trunk in which the fibres are still of small diameter and contain a number of fusiform swellings along their course. There are very few regenerating collaterals, however. Compare this trunk with the nerve trunk shown in fig. 20 taken from a preparation 12 weeks after crushing of the dorsal ear nerve. This photomicrograph was taken 5 weeks after crushing of the main dorsal ear nerve.

Fig. 16 (×475). Shows a maturing nerve fibre which had presumably met with obstructions at two places along its course. The photomicrograph was taken 5 weeks after crushing of the main dorsal ear nerve.

Fig. 17 (×1000). Shows also a maturing nerve fibre which had probably met with obstruction during the course of regeneration. The photomicrograph was taken 5 weeks after crushing of the main dorsal ear nerve.

Fig. 18 (×900). Shows a maturing nerve fibre in which one of the sprouts has swollen considerably. A collateral of the same fibre joins the swelling which then gives rise to three further fibres. Compare with fig. 17. The photomicrograph was taken 5 weeks after crushing of the main dorsal ear nerve.

Fig. 19 (×900). Shows the outline of a myelin sheath surrounding a regenerated nerve fibre. The irregular outline of the myelin sheath indicates that the fibre has commenced to degenerate. The reason for the staining of the Schwann nucleus is not clear. Such appearances are very rare. The photomicrograph was taken 5 weeks after crushing of the main dorsal ear nerve.

Fig. 20 (×600). Shows myelinated fibres in a small nerve trunk taken from a preparation 12 weeks after crushing of the main dorsal ear nerve. Compare with fig. 15.

Fig. 21 (×950). Shows a regenerated fibre in the cutaneous nerve plexus surrounded by a faintly outlined myelin sheath. The axis cylinder still contains swellings along its course. A node of Ranvier is faintly indicated at the top of the picture, which was taken 5 weeks after crushing of the main dorsal ear nerve.

PLATE 3

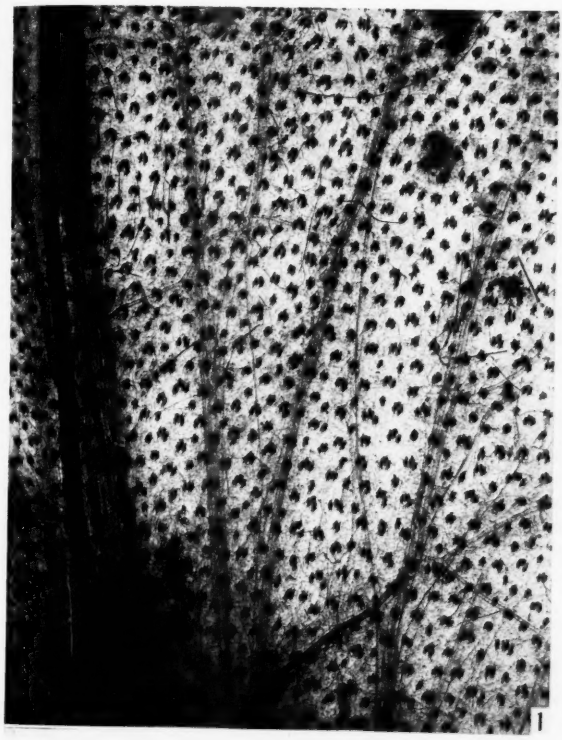
Fig. 22 (×575). Shows regenerating nerve fibres approaching the epithelium where nerve nets are starting to form. It is clear that the fine fibres are accompanied by prolongations of the Schwann cytoplasm. The photomicrograph was taken 5 weeks after crushing of the main dorsal ear nerve.

Fig. 23 (×1000). Shows a fine regenerating nerve fibre passing along the wall of a capillary blood vessel. The fibre is not yet beaded. The faint outline of a neighbouring capillary can be seen on the right of the photomicrograph, which was taken 5 weeks after crushing of the main dorsal ear nerve.

Fig. 24 (×550). Shows a regenerating nerve net in the wall of a small artery. The Schwann nuclei can be clearly seen and the prolongations of Schwann cytoplasm can be traced for some distance along the course of the fine net fibres, the majority of which, although irregular, are not beaded in the normal manner. Compare with fig. 26. The photomicrograph was taken 5 weeks after crushing of the main dorsal ear nerve.

Fig. 25 (×350). Shows a number of so-called 'Langerhans cells' just beneath the germinal layer of the epithelium. Fine nerve fibres can be seen to approach the processes of these cells which apparently lie in series with Schwann cells. *L*, Langerhans cell; *S*, Schwann cell nucleus; *N*, fine nerve fibres passing along the processes of the 'Langerhans cells'. The photomicrograph was taken 5 weeks after crushing of the main dorsal ear nerve.

Fig. 26 (×550). Shows a further stage in the formation of nerve nets in the wall of a small artery, the finer fibres are clearly beaded and the Schwann nuclei are no longer stained. The photomicrograph was taken 5 weeks after crushing of the main dorsal ear nerve.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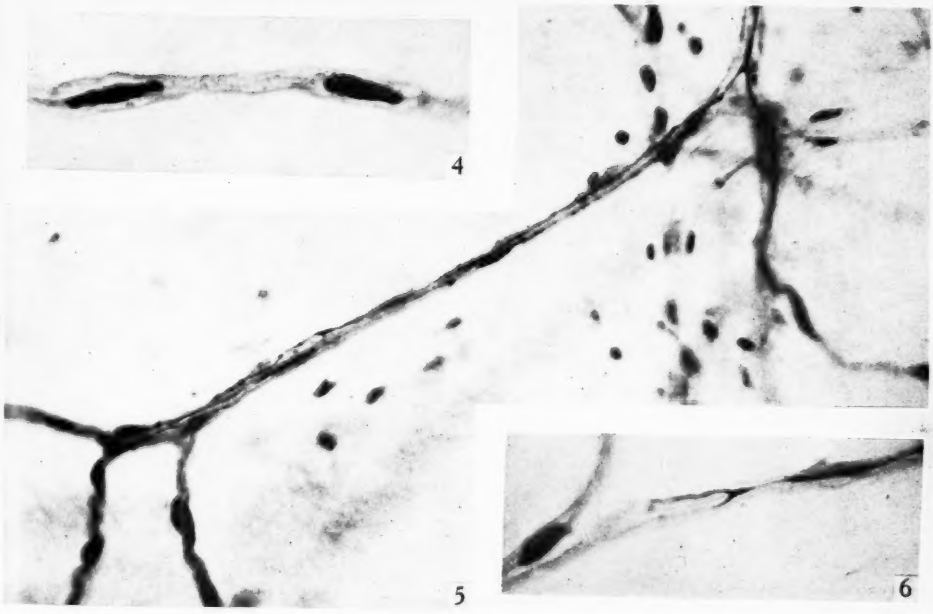
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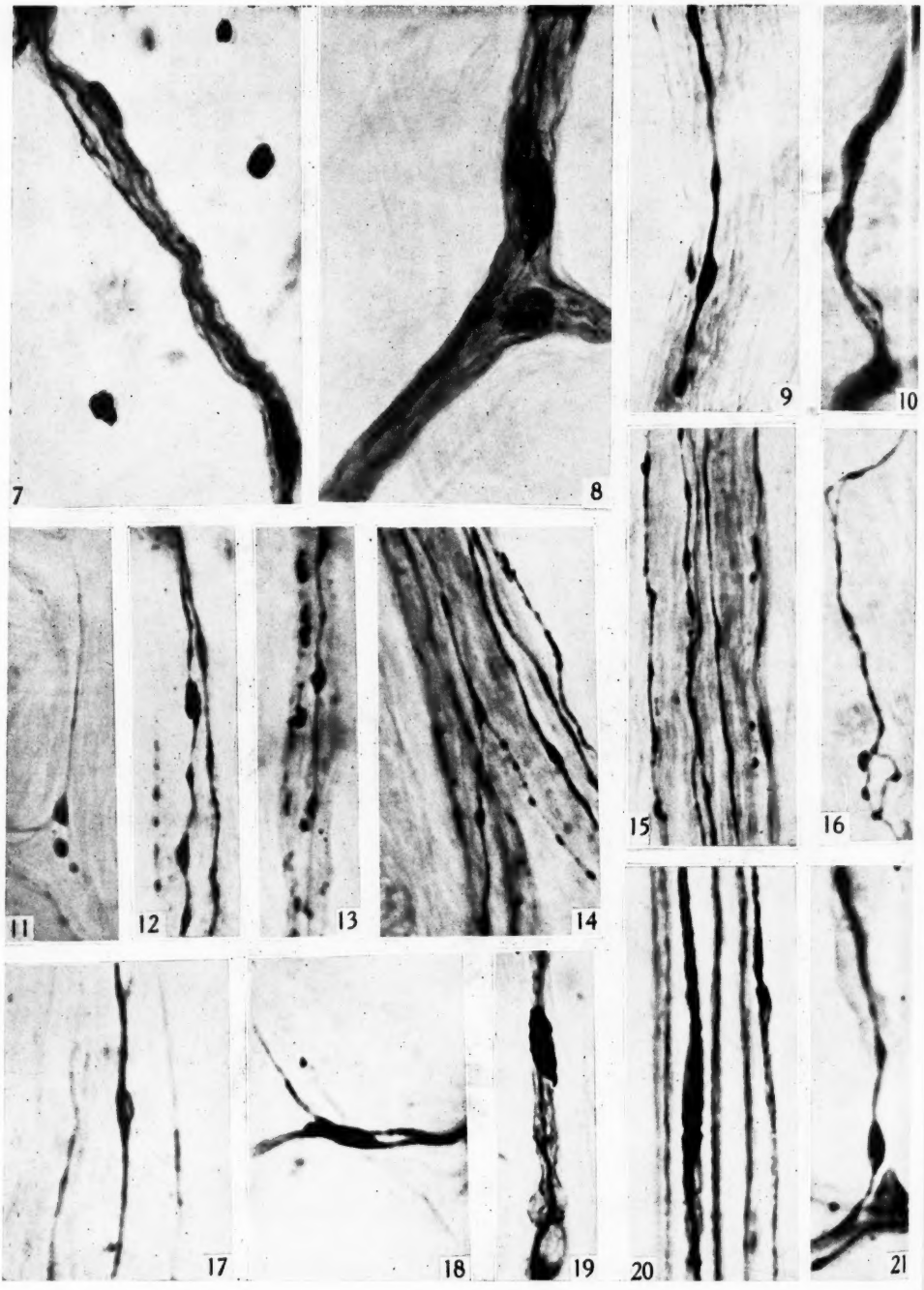
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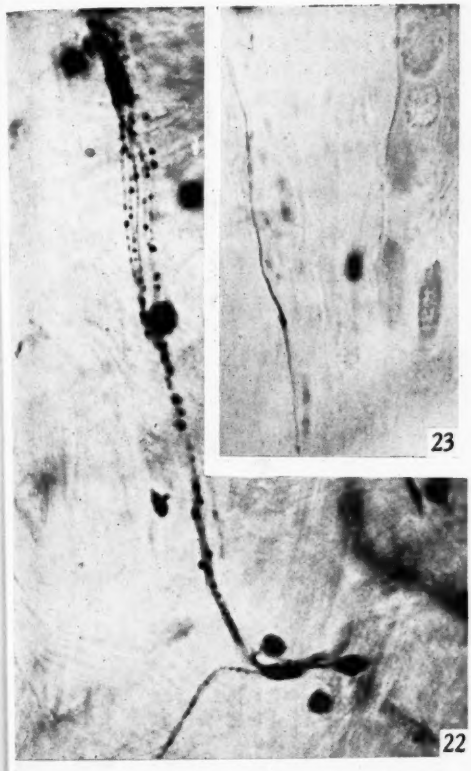

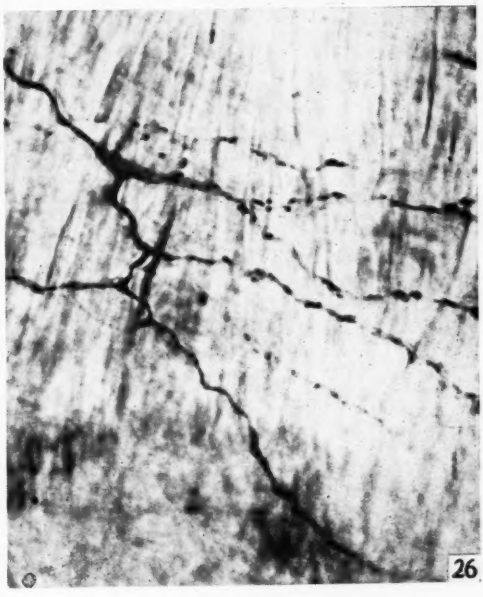

UNIV. OF MICH.

NERVE REGENERATION AFTER IMMEDIATE AND DELAYED SUTURE

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INTRODUCTION

Studies of the histology of nervous regeneration have mostly been confined to the period immediately following a primary suture. But since progressive changes take place in an uninnervated peripheral stump, the conditions which new fibres will meet when growing into it will vary according to the duration of the previous degeneration. Surgeons have often emphasized the desirability of early operation on unrecovered nerve lesions: Foerster (1929) found that in cases operated more than 6 months after injury the recoveries obtained were less successful than those after earlier operation. However, little attempt has been made to discover to what extent this failure is due to changes in the nerve itself rather than to progressive changes in the denervated end-organ.

The experiments here reported were made in order to provide a thorough study of the late stages of nerve degeneration and of re-innervation after varying periods of degeneration, and thus to assist in deciding how long it is permissible to delay before operating on unrecovered lesions. Failure of recovery as a result of changes in the damaged nerve may be due to changes (*a*) in the power of the central stump to put

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out new fibres, (*b*) in the ability of the peripheral stump to receive them and to recreate a normal nerve, and (*c*) in the success of the union between the two stumps at the point of suture.

The proliferation of the Schwann cells in degenerating nerve and the increase in volume of their cytoplasm have been described by many workers (Ranvier, 1878; v. Büngner, 1891; Howell & Huber, 1892; Nageotte, 1932; Cajal, 1928; Boeke, 1935). Very many others have referred to the 'syncytial bands of Büngner', though there is some confusion as to the nature of the objects to which v. Büngner's name is attached. He himself conceived them as rods of cytoplasm in which the new axones appear during regeneration. Boeke (1935) and Bielschowsky (1935) consider that the new axones grow through the solid bands; Cajal (1928) supposed that the bands, being formed from the collapse of the tubular Schwann cell, have at least a virtual lumen, through which the axones grow. Dustin (1910) tended to the view that the axones grow along the surface of the bands, and this we shall find to be correct.

Even greater confusion exists about the fate of the walls of the tubes which contain the 'bands'. We shall see that it is these walls which maintain the pattern of the degenerated stump, make it permeable to new fibres, and also restrict the growth of the latter and determine their final diameter. Although the importance of the 'neurilemma' as a covering to the normal nerve fibre is stressed in every text-book of histology, its relations to the tube walls in degeneration are unknown.

Much of this confusion arises because of ignorance about the nature of the normal nerve sheaths and uncertainty as to their nomenclature. In an attempt to solve the problems we shall first discuss the structure of the sheaths of normal myelinated fibres, then describe the changes in the Schwann cells, neurilemma and endoneurium in degeneration, following them up to the later stages, of which existing descriptions are particularly incomplete. We shall then describe the process of re-innervation after short and long periods of degeneration, and give the results of experiments designed to examine the effect of the factors already listed on the success of regeneration after secondary suture.

MATERIAL AND EXPERIMENTAL METHODS

The experiments have mostly been with rabbits, but we have been able to make comparison with material removed by Prof. H. J. Seddon and Mr W. B. Highet at operation on cases of peripheral nerve injuries in man. We owe them our thanks not only for this material but also for much helpful discussion. It is not possible with human material to make so exact an analysis of degeneration and regeneration as with experimental animals, because after a peripheral nerve lesion the material removed at operation includes a peripheral stump which has degenerated for a known time but which has usually been to some extent re-innervated for an uncertain period. However, many conclusions drawn from the rabbit material have been verified in man, and we have also examined experimental material from dogs and cats.

In the rabbits the operations have mainly been on the tibial nerve, but the peroneal has also been used, and the latter has the advantage that recovery of its motor functions can readily be tested by looking for reflex spreading of the toes (Gutmann, 1942). The three parts of the sciatic trunk can be separated up to the head of the femur (though the sural sends a branch to the tibial in the middle of the thigh), and it is thus possible to leave the sural intact while operating on either or both of the other

two divisions. This precaution is very useful in experiments of long duration, for so long as the sensory fibres to the heel carried by the sural are intact, 'trophic' sores of the foot are much reduced. In this way animals with severed nerves have been kept in good health for over 18 months.

All operations were performed aseptically under nembutal and ether anaesthesia. The experiments fall into the following four series:

(1) *Peripheral stumps degenerated for various times.* These were obtained by resection of the tibial nerve for most of the length of the thigh, usually with the further precaution of injection of an inhibitor such as formaldehyde or gentian violet into the central stump (Guttmann & Medawar, 1942). In most cases this procedure was successful in preventing union of the stumps and re-innervation of the periphery.

(2) *Primary sutures.* These were made to study the process of re-innervation. The suture was made by the application of plasma (Young & Medawar, 1940).[1] When the tibial is cut, the ends always retract, but with practice and the use of fine watchmaker's forceps they can be brought together again and successful and uniform sutures obtained throughout a series of experiments.

(3) *Secondary sutures.* In order to obtain peripheral stumps which had degenerated for various periods of time it was necessary to follow the same procedure as in series 1. But after this the tibial central and peripheral stumps are so widely separated that suture is impossible. This difficulty was met by suturing the central stump of the freshly cut peroneal nerve into the distal stump of the degenerated tibial. Similarly, in the experiments to test changes in the power of outgrowth of the central stump after section, the neuroma on the tibial was removed and the nerve sutured on to the peripheral stump of the freshly cut peroneal.

These procedures have the disadvantage that normal functional recovery cannot be tested, but they have the advantage that uniform sutures without any tension can always be made. Further, it is the only method by which changes in the regenerative capacity of central and peripheral stumps can be separately analysed (see Kilvington, 1912).

(4) *Outgrowth from the peripheral stump after various times of degeneration.* It is well known that when a peripheral stump is left far from a central stump strands of Schwann cells grow out from it forming a 'peripheral glioma' or 'Schwannoma'. It seems very likely that this peripheral outgrowth is of great importance in ensuring a satisfactory union of the stumps when nerves are sutured, even when their apposition is optimal. So we prepared, as in series 1, a series of peripheral stumps degenerated for various times and then cut them and left them without suture to determine whether the Schwann cells progressively lose their power to grow out and thus to make a satisfactory union at suture.

HISTOLOGICAL METHODS

All material was removed at biopsy operations. The pieces of nerve were kept extended under normal tension on cardboard, and fixed at once. The selection of histological methods is not easy, for the distribution and arrangement of the cytoplasm of the Schwann and mesodermal cells in the degenerating nerve can only be studied after

[1] We are very grateful to Mr P. B. Medawar for preparing the concentrated plasma with which all the sutures were made. Without the help of this method the whole investigation would have been very much more difficult, and parts of it impossible.

the best cytological fixation, such as is given by Flemming's fluid, and in thin sections (4 μ) which can only be obtained after paraffin embedding. Bouin's fluid was much less satisfactory than Flemming's and produced serious shrinkages. Unfortunately there is no method by which fine nerve fibres can be demonstrated after Flemming or any equally good fixation, and there is no doubt that many of the conclusions reached by early workers are unreliable, being produced by too great reliance on the use of special fixatives for the silver impregnation of axones. We have made much use of the method of Bodian (1936) on material fixed in his alcohol-formol-acetic mixture no. 2 (Bodian, 1937), but the appearances have been checked by reference to better fixed material. Material impregnated by Bodian's method can be counterstained with Masson's haematoxylin, Ponceau-fuchsin, light green, and in such preparations the protoplasm of Schwann cells is often well demonstrated by a purplish brown tint produced by a combination of the red stain with an incomplete differentiation of the haematoxylin, though unfortunately it is usually much shrunk by the fixation.

We have not employed any of the existing 'specific' methods for the staining of Schwann cells. Bailey & Hermann (1938) had no success with the methods of Nageotte, and the disadvantages of frozen sections discouraged us from the use of modified Hortega methods such as that employed by Murray, Stout & Bradley (1940).

Therefore, for the demonstration of the unshrunken protoplasm of Schwann cells we have found Flemming's fluid most satisfactory. The mixture we used was: chromic acid, 1 % 15 c.c.; osmium tetroxide, 2 % 4 c.c.; glacial acetic acid, 5 drops. Flemming material was counterstained with Masson's light green trichrome and with safranin and light green. Flemming without acetic was tried, and although the fixation of cytoplasm seems equally good, staining is more difficult. Fixation in Zenker's fluid followed by staining in phosphotungstic-haematoxylin by Mallory's method shows the cytoplasm of Schwann cells quite well in degeneration, and has the advantage that myelin remains are also visible.

In our extensive use of Bodian's method on material fixed in his alcohol-acetic-formol mixture and in formol saline we have often obtained preparations in which the cytoplasm of Schwann cells is sharply impregnated. With alcohol-fixed material this impregnation is usually found when the preparations are reddish in colour rather than blue-black, though these red preparations must not be confused with those given when some of the reducing solution is carried over on the slide into the toning bath. We have made many experiments with the method, but we have not been able to trace the variable that causes the method to give sometimes a sharp black impregnation of axones against a clear background, and sometimes an impregnation of Schwann cytoplasm, with the axones more or less well demonstrated also.

Recently we have found that the surest way of obtaining Schwann cell impregnation in degeneration is as follows: Mounted sections of formol-fixed material are impregnated overnight at 37° C. in 0·5 % aqueous Protargol (no copper is added to the bath). The sections are then treated by reduction with hydroquinone, toning, and a second reduction exactly as described in a silver method for axones already published, commencing at stage 8 (Holmes, 1942). In the resulting preparations Schwann nuclei as well as other nuclei in the section are darkly stained. The cytoplasm of the Schwann cells, although shrunken by fixation and embedding, stands out as blue-black filamentous strands (see Pl. 4, figs. 24, 26 and 29).

But it must be pointed out that many of our Schwann cell preparations have been

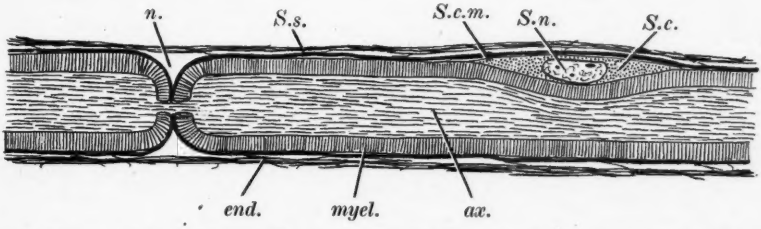
made by Bodian's original method, and we have not yet had sufficient experience with the new variant to say whether it can be used as a 'specific method for Schwann cells' under all circumstances.

Not the least of the difficulties of Bodian's method arises from variations in the composition of the Protargol, a colloidal silver preparation. We have used the Bayer brand of German manufacture and the American product of the Winthrop chemical company. Other substances marketed under the same name have not proved satisfactory.

For the demonstration of the myelin sheaths, formol-fixed material was mordanted for 14 days with 3% aqueous potassium dichromate and embedded in wax. The sections were stained overnight at 37° C. in Kulschitsky's haematoxylin, differentiated by Pal's method and counterstained with alum carmine.

Embedding has been through cedar-wood oil to wax, and usually transverse and longitudinal sections were cut from each block. We found that careful paraffin embedding need not produce appreciable distortion by differential shrinkage, even of the most delicate cytoplasm.

We owe our thanks to Mr James Armstrong for the very valuable work he has done in caring for the animals, and especially in dealing with such a large body of histological material.



Text-fig. 1. Diagram of a normal nerve fibre as it is revealed by methods here adopted. Based on tracings from the fibres shown in Pl. 1, fig. 1. The Schwann nucleus lies nearer to the node than it would usually do, but otherwise the proportions are correct. *ax.* axone; *end.* endoneurium; *myel.* myelin sheath; *n.* node; *S.c.* Schwann cytoplasm; *S.c.m.* Schwann cell membrane; *S.n.* Schwann nucleus; *S.s.* Schwann sheath (neurilemma).

THE SHEATHS OF THE NORMAL NERVE FIBRE

There is as yet no wholly satisfactory account of the relationships of the neurilemma, the Schwann cells and the endoneurium in a normal nerve fibre. In our best fixed material we see around each Schwann nucleus a small mass of cytoplasm (Pl. 1, figs. 1, 5 and 6), but we have not been able to convince ourselves that this layer extends over the whole of the myelin sheath. Nemiloff (1910), Doinikow (1911), Nageotte (1932), Cajal (1933), Rodríguez-Pérez (1934) and others have given figures showing a network of material all over the myelin, and they claim that this is the Schwann protoplasm, and some of them that it also extends within the myelin layer. But the appearances produced by their various methods are very different, and they do not demonstrate the 'protoplasmic nature' of the layer. We cannot deny the possibility that there is such a fine sheet of protoplasm, perhaps fenestrated, over the myelin, particularly as Doinikow said that it was not demonstrable in the rabbit on which most of our cytological work has been done. Sometimes, as in Pl. 1, fig. 1, there appears to be a definite edge or end to the Schwann cytoplasm, as if it does not extend

over the whole fibre. On the other hand, some of the appearances seen during degeneration can hardly be interpreted except on the assumption of a layer of Schwann protoplasm covering the myelin (Pl. 1, fig. 4 and Pl. 2, fig. 12).

If such a layer exists in rabbits' nerves it must be less than 1 μ thick, since it does not show, except near the nucleus, in transverse or longitudinal sections after Flemming fixation, which gives optimal preservation of the myelin and Schwann protoplasm (Pl. 1, figs. 5, 6). Over most of the surface of the fibre, therefore, the visible boundary adjoining the myelin is that clear membrane which Schwann first described (Pl. 1, figs. 1, 2, 5, 6; Pl. 2, fig. 7). It turns in at the nodes and is distinguished from the endoneurium outside it by being smooth and continuous rather than obviously fibrous. It often stains rather like collagen but may appear brown rather than green with Masson's light green trichrome stain after Zenker or Flemming fixation. This membrane, the sheath of Schwann, is often called the neurilemma, though this term would have puzzled Schwann. For in his time, and by some more recent authors (e.g. Cajal, 1928), the word neurilemma was used for the epineural and perineural connective tissue of the nerve trunk, and Schwann specifically stated that his sheath was distinct from the neurilemma in this sense. However, the term has been so widely used as a synonym for the sheath of Schwann that it is probably justifiable to retain it (see Young, 1942).

Outside the Schwann sheath lies the connective tissue which is definitely collagenous and does not turn in at the nodes (Pl. 1, figs. 5, 6; Pl. 2, fig. 7). This includes the sheath described by Key & Retzius (1873) and constitutes the endoneurium. To this endoneural connective tissue the term 'sheath of Henle' is often applied (see Ranvier, 1878).

There are, therefore, certainly three easily distinguishable entities outside the myelin: the Schwann cell, which may or may not cover the whole fibre, the Schwann membrane or sheath, alias neurilemma, and the sheath of Key and Retzius. Unfortunately, we still do not know enough about the chemical and physical natures or histogenesis of these layers to be able to devise an entirely satisfactory terminology. Even their appearance varies very much with the method of treatment.

Plenk (1934) holds that the situation can be described by saying that there is an inner endoneurium, which may be called the sheath of Plenk and Laidlaw, composed of a network of fine argyrophil fibres; and an outer endoneurium or sheath of Key and Retzius, of longitudinal collagen fibres. As he could not simultaneously demonstrate what is here called the Schwann sheath and the inner endoneurium Plenk believed the two to be identical. But this cannot be considered to be proved and we reluctantly conclude that it is premature to adopt the attractive solution of calling the neurilemma and the sheath of Key and Retzius the inner and outer endoneurium respectively. One danger in adopting such a terminology is that it may eventually be shown that the 'inner endoneurium' is a product of the Schwann cell, a view that has wide implications in neuropathology (Masson, 1932).

In any case it is most desirable to avoid confusion between the protoplasmic Schwann cell and the membrane here called neurilemma. It is therefore not desirable to use Schwann sheath as synonymous with Schwann cell (cf. Foot, 1940). It is the protoplasm of the Schwann cell which in the degenerated state forms the protoplasmic Schwann bands or bands of v. Büngner. The terms Schwann sheath or Schwann membrane should be used, if at all, as a synonym for 'neurilemma'. When Schwann

first described his membrane he implied that it was the outer membrane of the Schwann cell. But it may be presumed that he did not mean 'cell membrane' in the sense of modern general physiology. Though it is true that the Schwann sheath lies immediately outside the Schwann cell the preparations do not suggest that it constitutes the 'cell membrane' of the latter in the modern sense (Pl. 1, fig. 1 and Text-fig. 1).

1. *Progressive changes in the peripheral stump*

Our study of the degeneration of the peripheral stump is based mainly on nerves from forty-eight rabbits in which the stump was kept isolated, by the method described on p. 65, for periods from 6 days to 17 months. Many nerves from other rabbits have contributed details, and confirmation and comparison of many points have been made on human material, and on nerves from dogs and cats.

1·1. *Initial changes in the Schwann cells.* During the first 2 weeks after severance of a nerve, and while the axones and myelin in the peripheral stump are undergoing degeneration, the Schwann cells increase in number and amount of protoplasm. From the normal condition in which the protoplasm appears only as a small mass in the immediate neighbourhood of the nucleus, it extends and thickens till it lines a considerable part of the nerve tube, and then becomes a long strand as the myelin collapses (Pl. 1, figs. 3, 4; Pl. 2, figs. 12–14). Some stages suggest that this appearance of the Schwann protoplasm is the making manifest of a layer previously existing over the whole myelin surface (Pl. 1, fig. 4; Pl. 2, fig. 12). But even if there is such a layer it seems certain that in order to produce the voluminous strands of Schwann protoplasm seen 2 weeks after section there must be synthesis of fresh material. The nucleoli of the Schwann nuclei often become much more marked from the 4th day onwards, and this appearance may be related to the active process of protoplasmic synthesis (Pl. 1, fig. 4). However, it is also possible that this appearance is related to the division of the Schwann nuclei which is very abundant about the 9th day. Mitotic figures can still be seen after 15 days.

In the early stages of degeneration the myelin first disappears from the outer surface of the sheath (Pl. 2, figs. 9, 11), and it may be that this disintegration is brought about by enzymic activity of the Schwann cells. However, we have found little evidence that the Schwann cells are actively phagocytic, and none that they become converted into special phagocytic cells. Occasionally, while the Schwann protoplasm is increasing, granules of degeneration products may become included in their cytoplasm (Pl. 2, fig. 10). We shall not deal here with the detailed changes in the myelin, which have often been described. In places the myelin may retain its original form for a surprisingly long time (25 days or more), at least over short stretches.

1·2. *Macrophages.* It is probable that the removal of myelin and axone in degeneration takes place partly by autolysis and liquefaction, but the phagocytic activity of macrophages plays a large part in the process. Histiocytes from the connective tissue of the nerve, and from the walls of the blood vessels, can be seen in all stages of conversion into macrophages, and they move among the degenerating fibres and penetrate within them (Pl. 2, fig. 8). Here they ingest myelin and axone remains and become converted into typical foam cells. In our series of rabbits' nerves they appear in small numbers about the 7th day and become very numerous by the 15th day. They appear especially early in the region of the direct trauma to the nerve; in a

human nerve which had been crushed over a length of 5 mm., macrophages were abundant on both sides of the lesion 4 days later.

In rabbits the height of the macrophage invasion is reached during the 3rd week after the injury, and thereafter their number slowly declines. Macrophages laden with debris can be seen around the blood vessels, but their exact fate cannot be determined. These cells around the blood vessels never show the maximum of vacuolation characteristic of the fully developed foam cells within the fibres, and it may be that the latter never emigrate from the tubes but are themselves removed by a later invasion.

In the later stages of degeneration removal of the remaining macrophages takes place very slowly: some were still found in a stump which had been uninnervated for 17 months, and they often seem to have shrunk into small globules which are perhaps never removed at all (Pl. 3, fig. 15).

1·3. *The Schwann tubes and the movement of Schwann cells within them.* With the disappearance of the degeneration products there is a progressive decrease in the total diameter of the tubes which remain, and whose walls are made up of the neurilemma and endoneurium. The shrinkage takes place first between the groups of macrophages, giving the characteristic varicose appearance of a degenerate fibre (Pl. 2, fig. 8; Pl. 8, fig. 62). It is important to realize that a definite tube, representing the original fibre, persists throughout the process of degeneration. These tubes may conveniently be called *Schwann tubes*. They have sometimes wrongly been called the bands of Büngner, a term which should be restricted to the protoplasmic masses contained within the Schwann tubes. There has been much uncertainty as to the fate of the outer membranes of the nerve fibre during degeneration. Cajal (1928), after some hesitation, decided that what we here call the neurilemma disappears in degeneration. On the other hand, Nageotte (1932) and Masson (1932) believed that it becomes thickened by collagenization and merged into the endoneurium. We find that it persists throughout degeneration (Pl. 2, figs. 9, 11 and 12). The endoneurium outside it becomes thickened during the later stages and may in some cases seem to merge with the neurilemma, but in other preparations the latter appears quite distinct even after long periods of degeneration (Pl. 5, fig. 35; Pl. 6, figs. 46, 47).

As the myelin and axone disappear the walls of the tube collapse, shrinking down on to the Schwann protoplasm, which thus comes to fill the tube almost completely, except for the regions still occupied by macrophages and myelin remains (Pl. 2, figs. 11, 12). This filling of the tube may depend partly on further synthesis of Schwann protoplasm, but must also result from the squeezing down of the broader masses of cytoplasm which are found in the earlier stages. Thus already by the 15th day a considerable part of the length of the smaller fibres, and the regions between the macrophages in the larger fibres, are much narrower than the original tube and are filled with Schwann cytoplasm. However, this cytoplasm is not a continuous solid mass. Even with the best cytological techniques it not only shows signs of the well-known internal fibrils (Ranvier, 1878; v. Büngner, 1891), but it also appears that each Schwann cell to some extent retains its individuality, so that separate 'Schwann fibres' are formed. It is not easy to determine how this state of affairs arises without the use of means of continuous observation, but apparently after division each Schwann nucleus collects a portion of cytoplasm in the form of an elongated structure. Thus the tube comes to contain a number of these cells, often lying alongside each other (Pl. 2, figs. 13, 14; Pl. 3, fig. 23), the separation of the cells being particularly clear

when the 'fibre' of one cell runs past the nucleus of another (Pl. 2, fig. 14). It is exceedingly difficult in such delicate protoplasmic masses to determine to what extent the Schwann protoplasm is divided up in this way into separate 'fibres'. There is no doubt that in many cases two or more nuclei lie within a single body of protoplasm, unseparated by membranes (Pl. 4, fig. 31, *S.c.*). But we have been impressed by the fact that, even after Flemming fixation, lines appear which we can only interpret as indicating a certain degree of separation of the protoplasmic masses (Pl. 3, fig. 23).

The conclusion that the collapsed Schwann tubes are filled by elongated cells with some degree of independence is confirmed by a variety of appearances suggesting that the Schwann cells move actively within the old tube, seven when the earlier stages of proliferation have been passed. At either end of a macrophage or group of macrophages there are usually collected several Schwann cell nuclei (Pl. 3, fig. 20), the regions between the macrophages having long stretches which are almost devoid of them. Moreover, the ends of nuclei that face the macrophage are often flattened (Pl. 2, fig. 13; Pl. 3, figs. 15, 22), while those nuclei which have insinuated themselves between the macrophages and the wall of the tube do not show this flattening (Pl. 2, fig. 8). The collection together of numerous nuclei and the flattening of the ends of those that lie against the macrophages might be produced as a result of active pressure by either or all of (1) the Schwann cells, (2) the macrophages and (3) the neurilemma and endoneurium. That the macrophages exert pressure on the Schwann cells is shown by the fact that in some cases Schwann nuclei have both ends flattened (Pl. 3, fig. 21; Pl. 4, fig. 26). That the Schwann cells also migrate and exert pressure on the macrophages is suggested by the reverse appearance, namely, that as the cytoplasm of the macrophages shrinks the Schwann nuclei on either side of it remain closely flattened against it. When the remains of the macrophages are reduced to a persistent small droplet a Schwann nucleus can nearly always be found pressing against either side of it (Pl. 3, fig. 22; Pl. 4, fig. 26). It cannot be entirely excluded that these appearances are due to the pressure of the contracting endoneurium, but from our knowledge of other migratory activities of Schwann cells it is reasonable to suppose that they move within the Schwann tubes.

Table 1. *Lengths of Schwann cell outgrowth observed after leaving peripheral stumps isolated for various periods of time after section of the nerve*

Period of outgrowth days	Length of outgrowth mm.	Period of outgrowth days	Length of outgrowth mm.	Period of outgrowth days	Length of outgrowth mm.
15	1·2	15	1·7	30	5·0
15	0·8	15	1·4	42	3·5
15	2·0	25	3·4	53	3·6
15	1·7	25	2·0	54	3·5
15	1·5	25	2·2	83	4·5
15	0·7	25	2·0	218	3·0
15	2·4	25	1·4	484	3·1
15	0·5				

1·4. *Outgrowth of Schwann cells from peripheral stumps.* Active movement of the Schwann cells is also responsible for the well-known phenomenon of the outgrowth of Schwann cells from the end of the peripheral stump of a divided nerve, though the progressive contraction of the lumen of the Schwann tubes may also play a part in forcing the Schwann cells out of the end of the stump. In our series we have seen

very many examples of this outgrowth (Pl. 3, figs. 16–19; Pl. 9, fig. 71). It is not always easy to distinguish Schwann cells from fibroblasts, and we cannot give any final criteria for distinguishing the nuclei of the two cell types. Those of the Schwann cells are usually regular, and those of the fibroblasts irregular in outline. In the Schwann cell nuclei there are numerous small bodies while the fibroblast nuclei contain few and large granules. A more important and reliable distinction is the mode of growth of the cells, and hence their arrangement in the outgrowth. The Schwann cells usually grow out together in groups, making a strand several cells thick in which the cells are separated by little or no collagen, while the fibroblasts, even when longitudinally orientated, do not form such intimate strands and are usually separated by more collagen. Thus the columns of Schwann cells can be clearly distinguished after Mallory's stain as multinucleate red strands running amid the blue connective tissue. By these criteria the limits of Schwann cell outgrowth from a stump can be approximately determined, though it is not easy to locate the farthest limit reached by isolated strands growing out farther than the rest. Table 1 shows the lengths of the Schwann outgrowth from peripheral stumps at different intervals after section of the nerve, the central stump having been isolated and union prevented. The values are derived from measurement on longitudinal sections, and they are conservative estimates of the maximum distance of outgrowth, for they include only Schwann bands which could be positively identified. A few pioneering cells may well extend much farther. Also no correction has been included for the factor of shrinkage during fixation and embedding, so that the actual distances of outgrowth may be greater by perhaps 50 %. But, even so, the distances of outgrowth measured by histological methods would be less than those estimated by macroscopic study (Young, Holmes & Sanders, 1940). The explanation for this discrepancy is that at the end of a peripheral stump the fibrous tissue of the perineurium and epineurium grows out with even greater activity than the Schwann tissue. Thus an apparently large and diffuse 'Schwannoma' is often found microscopically to consist largely of fibrous tissue.

From Table 1 it can be seen that Schwann outgrowth is progressive in the first few weeks after nerve section, but from study of the peripheral stumps isolated for much longer periods we can state with certainty that it does not go on indefinitely. This condition contrasts with that described by Masson (1932) when a segment of nerve is isolated by two cuts, for then a Schwann outgrowth achieving a size many times that of the original nerve arises from both ends of the stump. We have found no comparable progressive proliferation from peripheral stumps of rabbits left after a single cut. Indeed, pieces removed from the tips of stumps left for long periods of degeneration often show very few Schwann cells extending into the scar. Possibly there is therefore some atrophy of the cells of the initial outgrowth.

Although Schwann outgrowth does not continue indefinitely we have often seen quite large gaps between central and peripheral stumps bridged when the nerves were left for a considerable length of time. Such bridges, of course, include some outgrowth from the central stump, but it is possible that they owe their development to an unusually lengthy outgrowth by the Schwann cells stimulated by the existence of a convenient pathway or line of stress in the operation site. In the later stages of the proliferation the Schwann cells in the outgrowing bands become separated by thick collagenous walls, but we have not been able to decide the origin of this collagen (see Masson, 1932).

In the earlier stages of outgrowth mitotic figures can be seen in the Schwann nuclei (Pl. 3, figs. 16, 18), so that the outgrowth is due to cell division as well as to continual wandering out of cells from the peripheral stumps. But in outgrowths later than 2 months or so mitoses are very rare. The nuclei in the outgrowths (and in the peripheral stump) often show curious appearances, sometimes having transverse partial divisions (Pl. 4, fig. 28). It would not be safe, however, to consider this appearance to be evidence of amitotic division of the Schwann cells.

It is probable that there is a good deal of anastomosis between the cytoplasms of the cells of these outgrowths, but the tissue is not a uniform 'Schwann syncytium' in the sense of a single mass of protoplasm like a slime fungus. Cracks and fissures between separate cells are very obvious, and these presumably provide surfaces over which new axones can creep (Pl. 3, figs. 16, 18).

1·5. *Late changes in an uninnervated peripheral stump.* There has been some dispute as to the later stages of degeneration of peripheral nerves which have been left uninnervated for long periods. It has sometimes been suggested that the stump becomes merely a mass of fibrous tissue incapable of receiving new axones, though Spielmeyer (1922) did not accept this view. It is certainly true, as Dustin pointed out (1917), that in the region of human nerve lesions the peripheral stump becomes fibrosed and incapable of receiving new fibres; but this hardly concerns us here, since it is a special phenomenon, and the resection of such fibrosed tissue is necessary before nerve suture (but see p. 77).

1·6. *Late changes in Schwann cells.* Our observations are based on 'sterile' stumps left for various periods up to 17 months. The later changes in the Schwann cells are simply a further evolution of those we have already discussed: indeed, some of the smaller Schwann tubes reach in a few weeks the condition of the larger ones after several months. The wall of the larger tubes contracts progressively for some months, so that the tubes become narrower. This is shown clearly by the change in form of the nuclei which become extraordinarily thin and elongated and after fixation often assume a characteristic sinuous appearance (Pl. 4, fig. 25).

The Schwann cytoplasm, meanwhile, is pressed out into long strands which often have very much the appearance of nerve fibres in silver preparations (Pl. 4, figs. 26, 29 and 31). In the smaller tubes there is no room for two nuclei to lie side by side, and the result is, therefore, a strand of protoplasm with nuclei at intervals along it (Pl. 4, fig. 31). In the larger tubes, however, the condition described in the earlier stages may persist, and the tube is occupied by two or more cells pressed side by side. The apposition between them is very close, and no open spaces are found in the tubes, but in both transverse and longitudinal sections it can be seen that cracks and fissures may exist between the cells (Pl. 4, fig. 27). The longitudinal fibrils within the Schwann cytoplasm can still be seen. They are often rather clearly demonstrated by Bodian's stain after formol fixation (Pl. 4, fig. 28), and fine blue striations are seen after fixation with Zenker and staining with phosphotungstic haematoxylin. But further discussion of the nature of this longitudinal organization of the cytoplasm must await the investigation of the birefringence and other physical characteristics of the cells.

After fixation with the alcohol-formol-acetic mixture transverse sections of the tubes often show the Schwann protoplasm shrunk to a number of fine threads distributed mainly at the periphery. These threads stain with Bodian's silver method, and brown with Masson's trichrome (Pl. 5, fig. 37). Thus in such preparations it usually appears

that there are considerable spaces in the Schwann tubes. But in well-fixed Flemming material it can be seen that the protoplasm fills the whole of the tube (Pl. 5, figs. 35, 36 and 38), so that the spaces seen after alcohol fixation must result from shrinkage, and occasionally a tube with well-preserved cytoplasm can be seen after alcohol fixation, while even in Flemming material the Schwann protoplasm in the centre of the nerve is often shrunken (Pl. 5, fig. 34). Often two nuclei can be seen lying side by side or end to end within an apparently homogeneous mass of cytoplasm (Pl. 5, fig. 35), but careful inspection of the well-fixed preparations shows the same signs of division into separate cells as was seen in the earlier stages.

The appearances seen with other fixatives also yield reliable evidence that the Schwann cells are to a considerable extent separate from each other. The fine drawn-out tip of one cell can often be seen ending against the side of another (Pl. 4, fig. 27). Still more convincing is the fact that some of the cells terminate in remarkable cytoplasmic balls or clubs, often of relatively very large dimensions (Pl. 6, figs. 40–42). These formations are presumably the result of the damming up of the Schwann cytoplasm by a macrophage at an earlier stage (Pl. 2, fig. 10). After the macrophage has been removed the Schwann cytoplasm is no longer in a sufficiently fluid state to allow the ball to become pressed out into a fibre. Sometimes, also, relatively broad masses of Schwann cytoplasm which are not terminal can be seen (Pl. 6, fig. 43).

We may conclude, therefore, that in the later stages of degeneration the Schwann tubes are entirely filled with the protoplasm of very elongated cells. Often these protoplasmic masses are multinucleate, but there are also cracks between them, especially in the larger tubes. Impregnation of these cells with silver by the method described on p. 66 after formol fixation, and sometimes by Bodian's method, shows a nerve stump full of very long fibres (Pl. 3, fig. 16; Pl. 4, figs. 24, 26, 29 and 30), and as each fibre may have a smooth outline extending over several millimetres they are often extremely difficult to distinguish from nerve fibres. That they are Schwann cells may be established by the fact that the protoplasm contains a nucleus, but if this is not included in the section, it is sometimes impossible even with the highest powers of the microscope to determine whether a fibre is an axone or a Schwann cell. It is probable that the difficulty is equally acute in the study of denervated stumps by other silver methods, and as pointed out elsewhere (Young, 1942), Cajal (1928) certainly made the confusion. Pl. 4, fig. 31, shows a partly re-innervated stump in which the more lightly stained Schwann cells and darker nerve fibres can just be distinguished, but it is very easy to understand that confusion between them might arise.

1·7. *Retrograde re-innervation of peripheral stumps.* Many workers have described normal nerve fibres in peripheral stumps after every precaution had been taken to prevent their re-innervation. Lugaro (1906), Perroncito (1907) and others produced evidence that such fibres do not arise by autogenous regeneration in the isolated stump. As the difficulty of distinguishing Schwann cells from axones is so great we thought it desirable to re-examine the question of the origin of such fibres. The problem is of considerable importance, for at operation on peripheral nerve lesions a sensory response obtained from the peripheral stump is usually taken as evidence that regenerating fibres have crossed the lesion and that further recovery may take place without operative interference. If sensory fibres can be found in peripheral stumps which are without doubt isolated from their central stumps, then the value of such an operative test is doubtful.

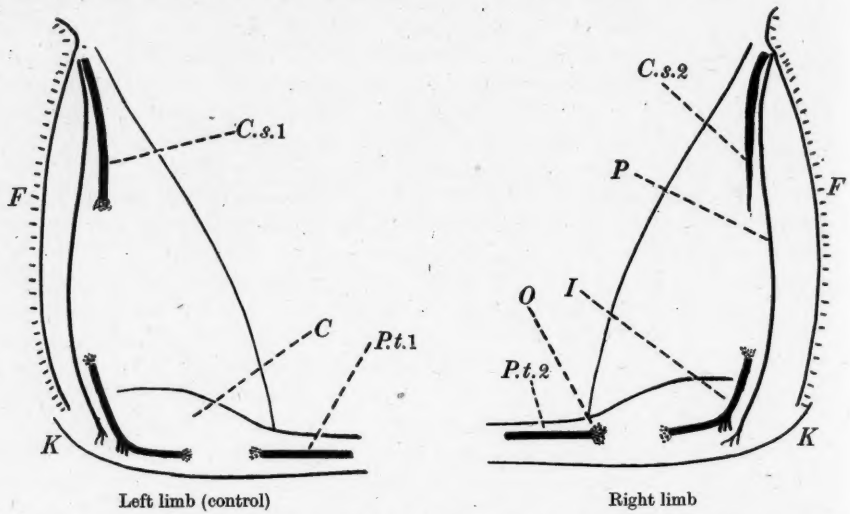
Early in our experiments we noticed that in animals in which special precautions had been taken to prevent union between the stumps, pinching or electrical stimulation of the peripheral stump after light anaesthesia always produced reflex responses. Such responses persisted even after the central end of the peripheral stump had been cut, to interrupt any fibres which might have bridged the gap in spite of all precautions. The fibres responsible for these anomalous responses must therefore enter the stump from the distribution of intact nerves, either by backward growth from the skin plexuses or by entry with the blood vessels. So far as we have been able to investigate their source systematically we find that these fibres enter from the blood vessels. Thus in rabbit 425 a length of 3·5 cm. was removed from the tibial nerve, and its central stump injected with osmium tetroxide. Sixteen months later the animal was anaesthetized and the nerve exposed. Pinching the peripheral stump was followed by reflex responses. A second pinch *central to* the first one then gave no response, but more peripheral pinches continued to do so. This shows clearly that the fibres are not derived from the central stump, but *ascend* the nerve. The peroneal nerve was then cut, but the responses were not abolished. The tibial peripheral stump was then completely exposed, but not freed from its bed, and a series of pinches made along it, beginning centrally. Reflex responses continued until the stretch of nerve just above the heel was separated from the posterior tibial artery with which it runs; after this no further responses could be obtained. Clearly the sensory fibres entered the nerve with the vessels at that point, for had they been derived from the skin plexuses the response would not have been abolished.

Similar evidence can be obtained by eliciting responses of this sort from a degenerated stretch of tibial nerve in the thigh, and then crushing the popliteal artery. No further responses can then be obtained from the nerve (see Gutmann *et al.* 1942). We conclude that few or no pain fibres run a retrograde course from the skin plexuses.

It should be noted that these retrograde fibres do not occur in a normal nerve. When a fresh nerve is exposed and cut no responses can be elicited by stimulation of the peripheral stump. After degeneration, therefore, the fibres from the blood vessels must be able to grow out into the old Schwann tubes. This has been shown to be so in an experiment in which an isolated tibial peripheral stump was prepared as above in the right hind-limb and left for 222 days (rabbit 644). After this time a second operation was performed, and the tip of the peripheral stump again cut, to interrupt any fibres from the central stump that might have found their way into it. The stump was also exposed peripherally in the shank, and a piece resected from it to interrupt any ascending fibres. The hitherto intact tibial nerve of the opposite limb was treated similarly. The animal was then left for a further 15 days to allow degeneration of any severed fibres, and outgrowth of any fibres which still remained connected with their cell bodies. Then the pieces indicated in Text-fig. 2 were removed and examined histologically. Fine nerve fibres were present in the most peripheral piece, *P.t.* 2, and they had grown out upwards from its end (Pl. 6, fig. 44). The other pieces removed contained no nerve fibres. Staining the peripheral piece with Weigert's method showed that of the fibres present a very few were medullated, but all were very small. Of course, no medullated fibres were present in the other pieces. *None of the pieces of the peripheral stump from the control side that had not been degenerated for* 222 *days contained any intact nerve fibres.*

In other similar cases we have found the same result: a few very fine medullated and unmedullated nerve fibres have grown into the long-degenerated stumps from the blood vessels, and they are sufficient to produce reflex responses on stimulation. We cannot, of course, say whether in man the sensation experienced on stimulation of such fibres would be referred to the region innervated by the normal nerve, but it seems likely that it might be deceptive in that way.

Considering that Schwann cells become structures very like nerve fibres, and that nerve fibres can enter an isolated stump from the periphery, it is not surprising that the autogenous theory of peripheral regeneration so long received the support of some experienced neurologists. They had more substantial grounds for their erroneous view than is often conceded by their opponents.



Text-fig. 2. Diagrammatic lateral views of the hind-limbs of rabbit 644 after exposure of the nerves in the thigh and shank at biopsy (see text). *C*, calf muscles; *C.s.*1, control central stump of tibial nerve 25 days after section; *C.s.*2, central stump inhibited with gentian violet 237 days previously; *F*, femur; *I*, segment of the tibial nerve at the knee, isolated for 15 days; *K*, knee; *O*, outgrowth of nerve fibres and Schwann cells from the most distal stump of the posterior tibial nerve; *P*, peroneal nerve; *P.t.*1, posterior tibial nerve on the control side; *P.t.*2, posterior tibial nerve on the long degenerated side.

1·8. *Late changes in the endoneurium and perineurium.* It would be interesting to investigate whether the decrease in diameter of the lumen of the Schwann tubes continues indefinitely. The diameter of the myelin sheath of the largest fibres in a normal rabbit tibial nerve after Flemming fixation is about 20 μ, while the largest Schwann tube in nerves similarly fixed after degeneration for from 337 to 514 days have a diameter of from 9 to 12 μ. Although we have not made careful measurements of the diameters at various times we have the impression that the tubes continually contract, but only very slowly. Thus the tubes in Pl. 10, fig. 72 are smaller than those in fig. 74. However, for the period we have studied we may conclude that the diameter of each tube is reduced by about a half. Each tube certainly remains as a definite entity. The complete process of 'degeneration' of the nerve therefore does not lead

to its conversion to a fibrous strand. Pl. 6, fig. 45, shows the pattern of Schwann tubes formed by the endoneurium after 1 month of degeneration, and although possibly some of the smallest tubes later become obliterated, the majority of them certainly persist for 18 months and probably very much longer.

As the Schwann tubes collapse their walls become considerably thicker. Both in normal and degenerated nerve the appearance of the endoneurium and neurilemma depends very much on the fixative used. In material fixed in Zenker's or Flemming's fluid the neurilemma is usually distinct as a thin sheath showing black or dark brown in haematoxylin preparations; while after Bouin or formol fixation it is not so clearly demonstrated. Similarly, in degenerated stumps the mode of fixation affects the appearance of the sheaths. After Zenker or Bouin fixation the collagen fibres of the endoneurium appear to have increased in number, while after formol or Flemming they seem to have undergone an individual thickening. But, whatever fixative is used, it is clear that there is a considerable increase in the amount of collagen between the fibres, making up to some extent the increase in the interfibrillary space produced by the collapse of the tubes. It is this collagen, no doubt, that gives the degenerate nerve its characteristic hard texture and resistance to cutting.

We have found that, close to the end of an isolated peripheral stump, collagenization of the Schwann tubes is more extensive than in the rest of the trunk, and it restricts the Schwann protoplasm to a very narrow band (Pl. 6, figs. 46, 47). It is interesting that such 'scarring' takes place even after the nerve has been cut cleanly under aseptic conditions. Its origin is obscure, but the collagen appears to lie inside the neurilemma, which is thus made visible as a very definite membrane. The Schwann protoplasm is reduced to a fine darkly staining strand at the centre of the tube, surrounded by a thick layer of substance staining green with Masson's stain though not obviously fibrous. It seems that this substance is collagen produced in the tube as a result of exposure or trauma at its cut surface.

This collagenization extends for several millimetres down stumps allowed to degenerate for a year or more. Since it restricts the lumen of the Schwann tube it presents a serious barrier to the ingrowth of new fibres (see p. 87 and Pl. 10, fig. 73). The likelihood of such scarring is a strong indication for removal of as great a length as possible from the end of long-degenerated peripheral stumps.

The perineurium, defined as the thin band of collagen which surrounds each nerve bundle in a normal trunk, shows no great thickening even after long degeneration (Pl. 4, fig. 33). The epineurium, which forms a sheath to the whole trunk and extends within it, separating the bundles with their individual perineuria, is somewhat thickened but not grossly so, and it contains many normal blood vessels.

The general appearance of a nerve that has been degenerated for a year and more is therefore not grossly dissimilar to a normal trunk. The Schwann tubes represent the original fibres, but are smaller than them. The endoneural spaces are larger, and filled with collagen, so that the whole nerve stains darkly with collagen stains. The bundles are smaller than normal, but their connective tissue sheaths are not greatly altered. The diameter of the individual bundles is reduced to about one-half, but the shrinkage of the whole nerve varies, reaching to a maximum of one-half.

2. *Re-innervation of the peripheral stump after immediate suture*

The observations described in this section were made on material from forty-nine rabbits in which the nerves were cut with scissors, sutured at once with plasma, and left for various periods from 15 to 194 days before biopsy. Some dog and human material has also been examined.

We are not concerned here with the processes by which union between the stumps is made and new axones put out by the severed fibres of the central stump, but only with what occurs in the peripheral stump when the fibres have reached it. After a good primary suture in the rabbit the first fibres appear in the peripheral Schwann tubes on about the 7th day, and the tips of the fastest axones advance along it at a rate calculated to be 3·5 mm. per day (Gutmann *et al.* 1942). We have studied the process of re-innervation mainly in longitudinal and transverse sections taken 1–2 cm. distal to the suture line on the 15th and 25th days after operation, but the later stages of innervation and medullation have also been examined. Since the rate of advance of the fibre tips is now accurately known we have been able to be certain that we were studying the relations of the axones to the Schwann cells in the very earliest stages of their association. It is very likely that many of the figures by which workers have purported to show the relations at these early stages in fact do not do so as they were not investigating conditions close to the tips of advancing axones.

2·1. *Advance of axones in the peripheral stump.* All the new fibres grow within the old tubes; those which attempt to force their way through the endoneurium or other connective sheaths are very soon blocked. Fibres running within the smaller tubes sometimes give the appearance of being in the endoneurium, but careful examination of longitudinal sections shows that every axone is within a tube containing a Schwann nucleus. It will be remembered that the Schwann tubes are almost completely filled by the Schwann cells and the extensive cytoplasm of the macrophages. It is not possible to demonstrate directly how the axones penetrate in relation to these protoplasmic masses, since the axones cannot be demonstrated in the material fixed with Flemming's fluid, which is the only method by which the cytoplasm is preserved without shrinkage and vacuolation. But, by comparing sections of well-fixed material with similar sections in which the axones are demonstrated and the Schwann protoplasm somewhat shrunken, it is possible to show with some certainty how the penetration takes place.

In nearly all cases the axones during the first few days after they have penetrated a given portion of a Schwann tube lie against the inner wall of the tube, between it and the Schwann cells and macrophages which occupy the centre (Pl. 7, figs. 48, 49, 50, 52, 53; Pl. 8, figs. 57, 58). At the level of a macrophage it is often possible to see clearly that the axones are flattened between its cytoplasm and the tube wall (Pl. 7, figs. 50, 52). In the regions between the macrophages, where the tube is occupied by the Schwann cells there is usually no doubt that at this early stage the fibres are lying between the Schwann cytoplasm and the wall (Pl. 7, figs. 48, 53). Occasionally fibres run down the centre of the tube and seem to be lying within the cytoplasm. It cannot therefore be absolutely excluded that axones do occasionally grow within the Schwann cytoplasm, but the fact that the great majority of the fibres lie at the edge of the tube strongly suggests that they grow over the surface of the Schwann cytoplasm, between the latter and the tube wall. Appearances such as those which have led

Boeke (1916) and Bielschowsky & Unger (1917) to suppose that the axones are growing down within vacuoles inside the Schwann cytoplasm may be the result of the misinterpretation of poorly fixed material, or of studying later stages when the fibres have become surrounded by Schwann protoplasm (p. 80).

On account of the possible presence of axone remains and other objects that may stain with Bodian's stain we have thought it well to include here a figure of an *uninnervated* stump, in order to prove that the objects showing in Pl. 7, figs. 48–53 and Pl. 8, figs. 57, 58, really are nerve fibres. Pl. 7, fig. 51, is from a region 100 mm. from a primary suture made 25 days previously, whereas the greatest distance at which fibres have ever been detected at this time is 80 mm. (Gutmann *et al.* 1942). The section is stained with Bodian and Masson and hence may be compared directly with Pl. 7, figs. 48–53. Pl. 7, figs. 49, 52, are indeed from sections taken 10 mm. from the same suture as Pl. 7, fig. 51. Pl. 8, fig. 56, is from the same region as Pl. 7, fig. 51, but is stained with Bodian's method alone and may therefore be directly compared with Pl. 8, figs. 57, 58. By such comparisons it is possible to say that at a point 10 mm. below a suture made 25 days previously numerous small fibres appear at the edges of the tubes and that these are not present in an unsutured stump or at this time at distances greater than at most 80 mm. from a suture.

Examination of longitudinal sections of material fixed in Bodian's alcohol fixative, stained by his method and counterstained with Masson's trichrome, often shows some quite well-fixed Schwann cells with nerve fibres running alongside them. This is most clearly shown in the re-innervation of stumps in which the internal organization of the nerve has been somewhat disturbed, as in Pl. 8, figs. 59–61, which are from a stored homograft. Here a new nerve fibre is seen running along the surface of several Schwann cells. This is especially clear at the bottom of Pl. 8, fig. 59, shown larger in Pl. 8, fig. 61, where the fibre apparently became blocked and sent out a lateral branch which left the surface of one Schwann cell (*S.c.a.*) and became attached to another (*S.c.b.*). However, the enlarged view of the upper portion of Pl. 8, fig. 59, which is shown in Pl. 8, fig. 60, shows the difficulty of making definite decisions on such points. Here it can be seen that there are various strands of Schwann protoplasm within the tube, and it cannot be decided whether the fibre is running within the somewhat shrunken cytoplasm of one cell or between that of separate cells. Pl. 8, fig. 62, shows that these conditions are not peculiar to grafted nerves, being a case from a stump re-innervated after simple suture. A single fibre can be seen running over the surface of the Schwann cells.

Pl. 7, figs. 54, 55, show in longitudinal section the numerous fibres running close to the wall of a single tube. Both are of the same tube, taken at different focal levels, in fig. 54 can be seen the fibres, and in fig. 55, taken at a lower focus, the macrophages around which they run. Fig. 55 also shows, in a neighbouring tube, two fibres running at opposite edges of a single small tube. Pl. 9, fig. 63, shows how difficult it is to be certain of the relationship of new fibres to the Schwann cells. A new fibre runs down the centre of a tube and might be held to be actually within the Schwann protoplasm. However, not only the innervated tube itself but other uninnervated tubes, shown in the figures above and below it, reveal clear fissures between separate Schwann cells, and it seems likely that the advancing fibres run in these.

In the early stages of degeneration the wall of the Schwann tube is not completely lined by Schwann cytoplasm, especially near the macrophages. Often therefore the

fibres may be without the support of Schwann protoplasm in their growth down the tube when they are passing between a macrophage and the tube wall (Pl. 7, figs. 50, 52).

It is usually supposed that the regenerating axones are provided with a bulb at their tip (see, for example, Nageotte, 1932, fig. 19), but we have rarely found these in our preparations of peripheral stumps during re-innervation, although, as has been pointed out, we have known with some exactness the point in the nerve at which the growing tips of the axones must occur. Occasionally small bulbs are formed where axones are obstructed by a macrophage, or even by a Schwann cell (Pl. 9, fig. 65), but it is probable that the growing tip is usually a tapering one, such as is seen in the earliest stages of the re-innervation of motor end-plates.

The new fibres entering a stump after primary suture are very numerous. As many as twenty have been counted around the walls of a single large tube, and ten may be present in quite a small tube. We have no evidence whether the numerous fibres in a single tube are the branches of a single axone, formed after it has entered the tube, or whether they are the processes of different nerve cells. The fibres do not run parallel to each other, and they frequently change their relative positions around the walls of the tube. It must be emphasized that these are separate fibres and not fibrils within a single axoplasm, and we agree with Bielschowsky (1935) in rejecting Boeke's interpretation that the various fibres in a single tube represent 'neurofibrils' which later become intimately associated with each other and acquire a common myelin sheath. We have seen no evidence that bifurcation and reunion of the axones, such as is found at the 'agonic' level in the cut central stump, occurs in the peripheral tubes. The individual fibres can be followed in longitudinal sections for considerable distances (Pl. 8, figs. 59, 62). However, it is worth emphasizing that they are excessively fine. The largest fibre shown on Pls. 7 and 8 is hardly above 1 μ in diameter.

Though the fibres at first lie on the surface of the Schwann cytoplasm they certainly later come to lie within it. Presumably as the axones increase in diameter (see below) they are forced into the protoplasm, which is known to have amoeboid powers, and thus flows around them. Difficulties of technique again make this process difficult to observe in fixed material. Indeed, at this stage the Schwann cytoplasm is particularly hard to demonstrate, presumably because it forms a thin sheet around the fibre. However, close to a point where a nerve had been crushed 25 days before, and at somewhat later stages after suture, it can be seen in both longitudinal and transverse sections that the axone lies within the Schwann cytoplasm, in clear distinction to its earlier position at its margin. At still later times it becomes obvious that the axones, or at least some of them, are enclosed within the Schwann protoplasm. Pl. 9, fig. 64, shows this in a preparation in which the myelin is partly preserved. When it is dissolved away, as in Pl. 10, fig. 75, the axone appears as a black dot at the centre of a 'vacuole' in the Schwann cytoplasm. This latter condition may have deceived workers who imagined that they were seeing the earliest stages of the growth of the axones within the bands.

2·2. *Increase in diameter and medullation of the fibres.* It would be most interesting to know the process by which some of the axones in the tube are selected for medullation (and, presumably, for function), while others disappear. It must in some way be connected with the rate of increase in diameter of the axones; even at quite early stages they are not all of equal size. Often there is one fibre much larger than the rest

which becomes surrounded by Schwann protoplasm and occupies the centre of the tube. But in many tubes it can be seen that more than one fibre lies within the Schwann protoplasm (Pl. 9, fig. 64; Pl. 10, fig. 75). It is not certain whether every fibre to be so included can become medullated if it acquires a sufficient diameter; certainly it is not infrequent for two or more fibres to medullate within one original tube (Pl. 9, fig. 64).

Presumably the whole course of maturation is determined by the increase in diameter of the new axones. This increase is certainly gradual and progressive along the course of the fibre. 10 mm. below a good suture made 15 days previously the largest fibres seen after shrinkage by fixation and embedding measured $1{\cdot}2\,\mu$, while the smallest were less than $0{\cdot}5\,\mu$ in diameter. The increase in diameter of the 'successful' fibres is rapid, and at 25 days the largest measure $2{\cdot}3\,\mu$; and at 100 days $7\,\mu$. Later the increase is slower. Sanders & Gutmann (1942) find that after 200 days a few of the fibres have reached the normal maximal diameter. This, measured as the total diameter of the myelin sheath in Weigert preparations, is $19{\cdot}8\,\mu$. (The equivalent maximal axone diameter of silver-stained material such as was used for the measurements given of the earlier stages would be approximately $10\,\mu$.) But even after this period most of the fibres are of less than normal diameter. Sanders & Gutmann found that the fibres in the central stump after a suture are also of reduced diameter, so it seems reasonable to suggest that some, perhaps all, of the increase of diameter of the peripheral regenerating axones is due to a downflowing of axoplasm from the central stump. Thus it may be that the axones in the peripheral stump which increase most rapidly in diameter will be those which are derived from central axones which have fewer other branches to supply. Such an arrangement would ensure that off-shoots of persistently branching fibres would be less successful than those from less branched axones, and the danger of abnormal functioning would be correspondingly reduced (see Young, 1942).

In a transverse section of a stump at 100 days there are far fewer axones in each tube than in the earlier stages (Pl. 9, fig. 66); some of those which have not increased in diameter have disappeared. In most of the tubes there is a single large fibre provided with a myelin sheath, and surrounded by a number of smaller fibres, apparently lying between its myelin and the tube wall. Two, three, or even more fibres may medullate within a single tube, and it may be that each acquires its own neurilemma and endoneurium, dividing up the original tube. But it is very difficult to make certain whether the transition from this condition to the normal regenerated nerve involves a true creation of several nerve fibres from within a single old tube, or whether some of the supernumerary fibres are later destroyed even though they have become to some extent myelinated. In the majority of the tubes there is only a single fibre left when medullation is well advanced.

We have not studied the processes by which myelin is laid down and nodes of Ranvier and other structures appear (see Gutner, 1936). Myelination seems to begin as soon as the axone is surrounded by Schwann cytoplasm: it can sometimes be seen immediately below the junction 15 days after suture. It certainly proceeds centrifugally along the fibres, but lags behind the tips of the axones along the peripheral stump (Gutmann *et al.* 1942).

3. *Regeneration after delayed suture*

This account is of three series of experiments by which we investigated separately three factors governing the success of regeneration after delayed suture. These are (1) changes in the power of the central stump to send out new axones, (2) changes in the peripheral stump which affect its power to receive these axones and to reconstitute a normal nerve, and (3) changes in the stumps which affect the quality of the junction made between them at secondary suture. The only similar experiments we have found described are those of Kilvington (1912) who went some way towards distinguishing the first two of these factors, but with whose conclusions we do not agree.

Table 2. *Test of power of outgrowth from a central stump severed again and sutured at various times after an initial cut. The distances of outgrowth were tested by pinching the peripheral stump. In the last column is shown the expected distance of regeneration, calculated from the data of Gutmann et al.* 1942

Animal	Time in days between injury and operation	Time in days between suture and biopsy	Distance of regeneration after delayed suture mm.	Distance of regeneration after control immediate suture mm.	Expected mm.
413	3	25	60	—	62
414	3	15	36	—	28
415	3	15	41	—	28
384	5	15	0	23	28
386	6	15	29	31	28
342	7	15	28	31	28
340	8	15	29	28	28
133	15	25	68	57	62
341	23	15	30	32	28
328	30	15	8	25	28
140	63	25	73	54	62
189	53	25	59	55	62
P6	103	15	30	—	28
P2	117	15	40	—	28
388	127	15	35	—	28
389	127	15	2	—	28
P4	277	25	76	69	62
P7	349	25	63	65	62

3·1. *The regenerative power of the central stump.* In eighteen animals the tibial nerve was cut high in the thigh and a gap left between the stumps so that a central neuroma was formed. Then after various periods ranging from 3 days to 11½ months a second operation was performed on each. The neuroma was removed from the central stump, and this was then joined by plasma suture to the distal stump of the peroneal nerve, which was freshly cut at a level convenient to allow suture without any tension. In most of the animals a control primary suture was performed at the same time on the other hind-limb, the tibial and peroneal nerves, hitherto intact, being cut, and the tibial central stump sutured to the peroneal distal stump. After a further period of 15 or 25 days the animal was again anaesthetized, and the distance reached by the regenerating fibres in the distal stump was tested by progressive pinching up the nerve (Young & Medawar, 1940). The value obtained for the distance of regeneration after secondary sutures of this type was compared with those found on the control sides of the animals, and also with the body of data on the rate of regeneration after primary

suture obtained from experimental work on other rabbits (Gutmann *et al.* 1942). It was thus possible within the limits imposed by this test to determine whether the outgrowth from a nerve which had been twice cut is of reduced vigour.

For convenience we may consider the results in two sets: those in which the two cuts were made within a few days of each other and those in which a longer delay was allowed.

When a central stump is cut a second time within 8 days of the original lesion outgrowth is as rapid as after a single cut and primary suture (Table 2). Indeed, in the seven animals which fall into this class all except two showed greater distances of outgrowth than were expected from the pooled results of control primary tibial-peroneal sutures. In animal 384 no innervation of the peripheral stump had taken place, but histological examination showed that the stumps had come apart. This did not occur in any of the controls, so it is an improbable event. We are not inclined to attach any significance to it as in all other animals in the series a good union was made. It is true, however, that secondary suture within a few days of the injury is made somewhat more difficult because the central stump is soft and diffuse, but the experiments leave no doubt that re-operation within a few days does not diminish the power of central outgrowth.

In the ten cases in which secondary suture was made at various times between 15 days and 11½ months there was no sign of any progressive decline in the distance reached by new fibres, and in all except two animals the distance of outgrowth was actually greater than was expected. The two exceptions, animals 328 and 389, show very low figures (Table 2). But in both cases the union was poor, and no great weight can be given to them: thus in 389 there was an unusual amount of retrograde degeneration in the central stump, presumably due to operative trauma, and there was a gap of about 2 mm. at the suture line.

But in all the other animals, even those in which the central stump had been left for nearly a year, the distance of outgrowth was normal; and we conclude that the power of outgrowth from a central stump does not diminish during the progressive formation of a neuroma.

The regenerating fibres present in the distal stump in these animals did not show any marked difference in number or diameter from those found in the control series. It would be interesting to examine the late stages of medullation after suture of an old central stump into a fresh peripheral one, but as the axones appear to increase in diameter at a normal rate during the early stages of regeneration there is no reason to believe that there is any other factor in the condition of the central stump which would interfere with recovery.

3·2. *The regenerative power of the peripheral stump.* In investigating the power of regeneration in peripheral stumps which have been allowed to degenerate for a long time we have examined not only the distances travelled by the regenerating axones within them, but also the later stages of medullation. The following account describes the experimental results. The histological observations on the re-innervation of stumps which have degenerated for a long time, forming a continuation of § 1 on the progressive changes in uninnervated nerve, are included in § 3·4 (p. 87).

Twenty-one animals have been used in the main series, the tibial peripheral stump being degenerated for times varying from 25 to 514 days (17 months), and union with the central stump being prevented by injection of the latter with some inhibiting

substance (p. 65). Then at a second operation the peroneal nerve was freshly cut, a small piece about 5 mm. long removed from the end of the tibial peripheral stump, and the peroneal central then joined with plasma to the tibial peripheral. In many cases a control primary suture of peroneal into tibial was made at the same time in the other hind-limb of the rabbit. In many of the animals the nerve was exposed after 15 or 25 days and the distance reached by the regenerating axones measured by the pinching method as before (Table 3). The table shows that of the five animals in which the nerve was degenerated for 25 days before suture four showed distances of regeneration equal or greater than those given by the control primary sutures. There is here therefore a distinct indication that either the junction is better made or fibres

Table 3. *Test of power of peripheral stump to receive new fibres after various periods of degeneration. See description of Table* 1

Animal	Days between lesion and suture	Days between suture and biopsy	Distance of regeneration after delayed suture mm.	Distance of regeneration after immediate suture mm.	Expected distance of regeneration mm.
790	25	15	40	43	28
793	25	15	38	22	28
794	25	15	39	30	28
805	25	15	31	34	28
806	25	15	31	22	28
82	33	25	66	61	62
150	38	25	65	74	62
201	45	11	13	11	11
170	53	15	23	—	28
124	54	25	49	—	62
175	91	25	47	63	62
84	126	15	33	—	28
211	150	25	15	—	62
179	236	15	26	—	28
168	241	15	32	—	28
297	309	25	25	—	62
P11	315	25	26	61	62
P8	331	25	32	59	62
P12	365	25	44	—	62
P10	365	25	67	—	62
P1	514	25	56	57	62

enter more freely into predegenerated stumps. We shall see that the former is probably the correct explanation.

Of the five animals in which the nerve was degenerated for periods between 33 and 54 days three showed slightly less than the distance of regeneration after primary suture, and three showed more than expected, but in none was the difference from normal great. After degeneration for this time, therefore, delayed is at least as effective as immediate suture.

On the other hand, of the eleven stumps degenerated more than 90 days, six showed a distance of outgrowth within expected range, and the remaining five showed only short distances of regeneration. A series of five failures is an altogether improbable number to be attributed to detached sutures or other accidents, as can be seen from the experiments listed in Table 2, and from the very many other primary sutures we have made. We must, therefore, conclude that some factor is operating in these degenerated stumps to reduce the rate of advance of regenerating fibres. But

this factor is not universal in its operation, and two of the longest degenerated stumps, P10 and P1, showed outgrowths well within the range of expectation from primary sutures. We must thus look for an influence which in some cases retards the growth of fibres after secondary suture.

3·3. *The quality of union between the stumps at secondary suture.* In searching for the reason for the poor outgrowth discussed in the last section we examined the suture regions in these animals histologically. In the animals in which the stumps had been degenerated up to 54 days excellent unions had been made, but in none of the animals degenerated for more than 90 days was the union of the same quality as that we have come to recognize after primary plasma suture. Pl. 9, fig. 67, is of a suture made into a stump that had been degenerated for 38 days: it shows a very good union. Pl. 9, fig. 68, shows that quite a good suture can be obtained after 150 days degeneration. In this animal fibres had penetrated less far into the stump than after a comparable primary suture, in spite of the closeness of the junction. Pl. 9, figs. 69, 70, show much less satisfactory sutures after degeneration of the peripheral stump for exactly one year in each case. The sutures had been made for 97 days before biopsy and these show that the union has not improved in intimacy even after a longer period. Even in the animal in which fibres had penetrated the full distance after 514 days' degeneration, examination shows that the suture, although close, has not effected an intimate union. In all these cases of suture into stumps degenerated for a long time, one obtains the impression that although the apposition between the stumps was close the union between them was not good. Instead of numerous strands running straight from one stump to the other, as in Pl. 9, fig. 67, there are at best irregular and criss-crossing fibres. In fact, the line of separation between the stumps remains sharp, instead of almost disappearing as it does after a good suture.

We conclude, therefore, that the poor union was not due to poor operative technique, except possibly in animals 179 and 297 in which we had difficulty in effecting the secondary suture, the peripheral stump being awkwardly low in the popliteal space, and embedded in fat. In the other cases the histological evidence showed that the apposition at the suture was good, but the union was bad.

3·31. *The activity of Schwann cells at secondary suture.* Since the migratory activities of Schwann cells from the cut ends of nerve stumps play a large part in effecting a union after suture, we investigated the possibility that the poor unions at secondary suture were due to a decline of the power of the Schwann cells to grow out of peripheral stumps which had degenerated for a long time. In ten animals degenerated stumps were prepared as in series 2 above by resection of a portion of the tibial nerve and inhibition of the central stump. At a second operation a piece about 5 mm. long was removed from the tip of the peripheral stump which was then left, without suture. At the same operation a piece was resected from the hitherto intact tibial nerve on the opposite side of the animal, and the peripheral stump left isolated. After a further period of from 9 to 25 days the animals were killed, and the tips of the two severed peripheral stumps removed. Longitudinal sections were cut of these pieces and of those removed at the earlier operation. It was thus possible to determine the distance and amount of Schwann outgrowth from a normal stump and from one degenerated for a long period. In Table 4 the criterion of the distance of Schwann outgrowth was the distance from the margin of the stump reached by the farthest identifiable strand of Schwann cells. Clearly this gives only a general idea of the vigour of the outgrowth,

because of the difficulty of identifying Schwann cells (p. 71), but the figures are reasonably accurate and comparable. Table 1 was obtained in a similar way and shows the distances of normal primary Schwann outgrowth from freshly cut stumps. Comparison of Tables 1 and 4 shows that after long periods of degeneration the power of Schwann cells to grow out from the stumps becomes very distinctly reduced.

But the results given in the tables also show that the most active outgrowth is given not by a freshly severed stump, but by one which has undergone a short period of predegeneration. Moreover, in the animals in which these longer distances are given the density of outgrowth was also correspondingly greater than from a freshly cut stump. Pl. 9, fig. 71, shows a very abundant growth found 15 days after severance of a nerve which had been predegenerated for 53 days.

It is somewhat surprising that there is any proliferation at all of Schwann cells when the nerve is cut a second time, for they have already gone through their degenerative change of mitosis and multiplication. The secondary outgrowth is not simply an emigration without multiplication, for mitoses can be seen in the secondary outgrowths (though they do not occur elsewhere in the twice cut stumps). Presumably the stimulus to cell division is confined to the traumatic region at the end of the nerve,

Table 4. *Lengths of Schwann cell outgrowth from peripheral stumps cut a second time after varying periods of degeneration*

Period of degeneration days	Period of outgrowth days	Length of outgrowth mm.	Period of degeneration days	Period of outgrowth days	Length of outgrowth mm.
9	30	3·5	222	15	0·5
31	25	2·5	236	15	0·5
53	15	1·4	241	15	0·3
53	25	3·3	309	25	0·2
126	15	0·8	484	9	0·4

but it is still remarkable that the Schwann cells not only retain their power of outgrowth but retain it to a greater extent than normal after a moderate amount of predegeneration.

The work of Abercrombie & Johnson (1942) provides a striking elaboration of these observations. They have investigated the migratory powers of Schwann cells in denervated nerve cultivated in vitro. They confirm Ingebrigtsen (1916) in showing that no emigration takes place from explanted normal nerves: from the stumps degenerated for 4 days and more the amount of outgrowth increases greatly, and it is at a maximum from stumps predegenerated for about 25 days. After this it falls to a plateau in stumps of about 50 days, and there is a gradual subsequent decline, though even after a year of predegeneration it never fell back to the condition of normal nerve, from which no outgrowth takes place.

These changes in the power of outgrowth of Schwann cells seem sufficient to account for the variable success of union at secondary sutures. After primary suture the Schwann cells begin to grow out after 4 days, probably as mitosis begins, and the migration during the succeeding days plays a large part in making the union. The fact that the most vigorous outgrowth is from nerve predegenerated 2–4 weeks suggests that secondary sutures made after this period would give the most successful of all junctions. As we have seen on p. 84 this is indeed the case.

This phenomenon of rapid outgrowth from peripheral stumps after short periods of predegeneration may well explain the success claimed for predegenerated nerve grafts (Ballance & Duel, 1932). Sanders & Young (1942) found that regenerating axones do not grow faster within predegenerated autografts than within fresh autografts, but that the delay at the suture line before the fibres enter the grafts is shorter for the predegenerated type. It would seem, therefore, that predegeneration does confer an advantage in grafting, by ensuring a more efficient and rapid union, though this was not the reason for which predegeneration was introduced.

The decline in Schwann outgrowth is very likely to be responsible for the formation of bad unions after very delayed secondary suture. It must be emphasized that although our investigations only tested directly the *distance* of regeneration in the stump, the poorer junctions certainly allow also a much smaller *number* of axones to reach the peripheral stump, with permanent effect on the likelihood of successful recovery. The decline of Schwann cell activity in the peripheral stump is thus a factor which indicates that it is unwise to delay suture for long periods if this can be avoided.

3·4. *Re-innervation of nerve after long periods of degeneration.* We have now to examine the process of penetration of new fibres into a stump which has degenerated for a long time, to discover whether this differs from the re-innervation of freshly cut stumps in any respect likely to impair the effectiveness of recovery. For this purpose we have had the material from all the animals listed in Table 3. Most of these were killed 25 days after suture, but to study the later stages of re-innervation some of them were left alive for up to 103 days after suture.

The chief differences between an uninnervated peripheral stump 15 days old and one of say 100 days are that in the latter the macrophages are much fewer, the diameter of the Schwann tubes has been reduced, and they are filled with Schwann protoplasm. Longitudinal and especially transverse sections (Pl. 10, figs. 73, 74) show that new fibres entering such an old stump all run within the Schwann tubes, just as they do in primary re-innervation. Pl. 10, fig. 72, shows a section similar to fig. 73 in all respects except that, as the former was 85 mm. from the suture made 25 days before, no nerve fibres are present. In transverse sections taken some 10 mm. below a secondary suture made 25 days previously, the new axones are mostly of much larger diameter than those seen after primary suture (compare Pls. 7, 8, figs. 48–58 with Pl. 10, figs. 73, 74). This may perhaps be due to the greater resistance which impedes their advance and results in the axoplasm flowing out from the central stumps, 'dilating' the axones more rapidly. So far as fixation difficulties allow, it is possible to say that there is no evidence that the axones grow out within the Schwann cytoplasm. In the early stages they usually occur at the periphery between the cytoplasm and the tube wall. However, even after 25 days, some of them give the appearance of being within the protoplasm and this is certainly the condition in later stages (Pl. 10, fig. 75).

The number of fibres in each tube is definitely less than after primary suture, even at the earliest stages of re-innervation, although in these experiments the source of the new axones was always a freshly cut normal nerve. This reduction of the number of fibres in the Schwann tubes may be due either to a failure of axones to reach the peripheral stump because of the poor Schwann outgrowth, or to the reduction in the size of the lumen of the tubes, and the increase of endoneural collagen. It seems likely that this second factor is the more important, for we saw signs of a reduction

of the number of axones in the peripheral tubes after a length of degeneration which should not have caused a serious decline in the power of outgrowth of the Schwann cells (Pl. 10, fig. 74). Pl. 10, fig. 73, shows that some of the Schwann tubes have become so reduced in diameter by collagenization that it is very difficult for new fibres to enter. Probably this restriction of the tubes would have been less severe somewhat farther from the injured surface (cf. Pl. 10, fig. 72), and this case emphasizes the need for adequate resection of the peripheral stump when making a suture after long delay.

The reduction in the number of fibres entering the peripheral stump will interfere with recovery by reducing the chance that successful reconnexions will be made. It might be expected, however, that the later development of the fibres in the stump, particularly their medullation, would not be interfered with, for each axone is well in contact with Schwann cytoplasm. Actually, a stump examined at any time after a long-delayed suture shows fewer and smaller medullated fibres than a corresponding stump after a primary suture (Pl. 10, figs. 76, 77). Twenty-five days after an immediate suture there is already often a good deal of medullation in the upper part of the peripheral stump, but after a suture delayed for 514 days no medullation at all was seen after the same period.

This delay in the increase in diameter and medullation of the fibres may be due to physical conditions within the Schwann tubes, whose thick walls may resist the expansion necessary for them to contain a full-sized medullated fibre, and it may be due also to some failure of the Schwann cells in synthesizing myelin. It can hardly be directly related to inadequate vascularization of the stump, as we have already described that an apparently ample vascular supply is present. In any case there is no doubt that at 3 months after suture the regeneration of the nerve is very much less complete if the peripheral stump had previously been allowed to degenerate for a long time. Pl. 4, figs. 32, 33, show that at this time the predegenerated stump still shows an excessive collagenization and Pl. 10, figs. 76, 77, that it shows inadequate medullation. Moreover, the threshold to faradic stimulation of the peripheral stump was found to be higher 3 months after delayed than after immediate suture.

After suture to an old peripheral stump there will be at best a considerably greater delay in return of normal function than after a primary suture. It is likely, though not proved, that in such cases the recovery can never, even slowly, become complete. For as has been pointed out (Young, 1942) optimal recovery presumably requires innervation by fibres of normal diameter and medullation. We do not yet know whether the much-contracted tubes of an old peripheral stump can be gradually expanded until they are of normal diameter, but there are reasons for suspecting that they may remain permanently small. Recent experiments in which spinal nerves were sutured into post-ganglionic sympathetic nerves have shown that medullated fibres form in the latter trunks, but they remain very small (Simpson & Young, unpublished). It is clear, therefore, that the size of the tube in the peripheral stump in which a new fibre finds itself imposes a certain limitation on its growth which may have the effect of permanently specifying its diameter. Examination of later stages of medullation is now being undertaken to test whether this is really the case.

DISCUSSION

Comparison of immediate and delayed suture

The final criterion of the effectiveness of immediate and delayed suture is the degree of functional recovery that they produce. Unfortunately, there are exceptional difficulties in making such a direct comparison experimentally. The device of crossed union of nerves is essential for the making of comparable sutures, and a full analysis of functional recovery after crossed union is difficult. Gutmann (1942) has partly overcome the difficulties by comparing primary and secondary suture of tibial into peroneal nerves in the rabbit. After this anastomosis there is usually some recovery of the toe-spreading reaction which is normally effected through the peroneal nerve. He found that in rabbits in which the peroneal had been degenerated before being sutured to a tibial central stump, the recovery was always slower than the primary crossed suture and was also less complete than on the control side. However, the effects only became severe when the peripheral stump had been degenerated for more than 6 months.

There is at present no body of data comparing the results of primary and secondary suture in man. Even if the clinical results of delayed suture were known, it is most unlikely that such data would allow analysis of the factors liable to affect recovery. There are at least six factors which may affect the success of secondary suture:

(1) The power of the central stumps to send out new fibres. We have seen that this power is not affected when the axones are cut twice, whether the axones are cut within a few days or after nearly a year from the original injury.

(2) The power of the stumps to unite. From the point of view of successful functional union this depends on the outgrowth of Schwann cells from the peripheral stump. This outgrowth is at its maximum not immediately after severance of a nerve, but during the period about 2–3 weeks later. We have evidence that unions made after this interval are somewhat more successful than primary sutures. For some time longer this power to make good unions remains high, but from about 100 days onwards it declines, and long-delayed unions are less likely to be successful than primary suture.

(3) The total diameter of the peripheral stump shrinks in the later stages of degeneration, sometimes by as much as a half: it is thus more difficult to effect a good suture, and the successful re-entry of fibres into the peripheral stump is prejudiced.

(4) The shrinkage of the lumen of the Schwann tubes results in fewer fibres entering each one, and presumably reduces the chances of successful reconnexions. But the distance reached by the most advanced regenerating fibres in these old tubes is sometimes not less than after primary suture, so that they can offer favourable pathways for new growth.

(5) After delayed suture medullation is delayed in the peripheral stump, and the fibres remain of small diameter for much longer than after primary suture. It may be that this condition persists indefinitely, and, if this is so, functional recovery must remain always of inferior quality after delayed suture.

(6) The factor of re-innervation of end-organs is of great importance. It is not included in the present study, but it is probable that motor end-plates and sensory end-organs gradually disappear in denervated tissues and, of course, there is profound atrophy of the muscle fibres themselves (see Tower, 1939). Methods have been pro-

posed for avoiding muscular atrophy, fixation of joints and other progressive changes, but the destruction of end-organs cannot be prevented by any known physical treatment. Even if new and satisfactory end-organs can be formed after delayed suture the process is likely to be much slower and less satisfactory functionally than the re-innervation of old end-plates and organs in the skin which follows primary suture.

We may conclude that the processes of union of stumps and the regeneration of a new functionally efficient stretch of nerve are less satisfactory after the longer periods of degeneration. Delay in suturing for 1 or 2 months does not prejudice the chances of recovery, and may indeed even improve them; longer delays, especially those greater than 5 or 6 months, produce conditions which at least are liable to retard recovery, and may permanently prevent its completion.

SUMMARY

1. During degeneration the pattern of a nerve is maintained by the endoneurium and neurilemma, a *Schwann tube* being formed in place of each original fibre. Each tube gradually contracts, finally reaching about half the diameter of the original fibre.

2. Macrophages enter these tubes and remove the remains of axones and myelin. They are abundant from about the 8th to the 25th day and thereafter disappear gradually, some persisting for a year or more.

3. The protoplasm of the Schwann cells increases in amount and after nuclear division elongated cells are found, lying side by side in the larger tubes.

4. In early stages the Schwann cells do not entirely fill the tubes. They gradually replace the macrophages and when the tube is fully contracted its whole cross-section is filled with fibrillar Schwann protoplasm, the nuclei becoming extremely elongated. Such Schwann cells may easily be mistaken for nerve fibres, especially when stained with silver.

5. In early stages the Schwann cells probably move within the tubes. Certainly they emerge at the end of the peripheral stump and are the main agent for leading fibres back into the old tubes.

6. Growth and mitotic division take place in the Schwann tissue which has migrated from the peripheral stump. The tip of the outgrowth was found to have reached to an average distance of 1·4 mm. in 15 days, 2·2 mm. in 25 days. But the outgrowth does not continue progressively if the stump is left uninnervated.

7. Nerve fibres usually grow into isolated degenerated peripheral stumps from the blood vessels. They remain very small and are rarely medullated, but are sufficient to produce a reflex response on stimulation and an outgrowth of nerve fibres from the *peripheral* end if the stump be again cut.

8. The endoneurium and perineurium of a long-degenerated stump become moderately but not excessively collagenized. The diameter of the whole nerve may decrease by as much as one-half.

9. When new fibres grow into a freshly cut peripheral stump they run along the inner walls of the Schwann tubes. Where possible they grow along the surfaces of Schwann cells, but do not at first lie within them.

10. As many as twenty fibres may enter a single tube. One or more, rarely several, of these increase in diameter, become surrounded by Schwann cell protoplasm and medullated. The others remain for a while around the periphery of the tube and presumably finally they atrophy.

11. The power of a central stump to send out new fibres is not reduced if it be severed a second time and then sutured, either within a week or after an interval as long as a year.

12. The power of a peripheral stump to put out Schwann cells is greatest if it be cut a second time some 2–3 weeks after its first severance. Thereafter this power declines, and unions made with peripheral stumps degenerated for more than 100 days are often unsatisfactory.

13. Once within a peripheral stump which has been degenerated for a long time, however, fibres may proceed as rapidly as into a freshly cut one.

14. Fewer fibres penetrate into a stump which has been degenerated for a long time than into a freshly cut one.

15. Medullation proceeds much more slowly after delayed suture.

16. There are therefore various factors, in addition to atrophy of the end-organs, which are likely to reduce the effectiveness of recovery when suture is made after a long delay.

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EXPLANATION OF PLATES

'Masson' indicates staining with Masson's light green trichrome method. 'Mallory' indicates Mallory's aniline blue trichrome. Except where otherwise stated the material was embedded in paraffin wax through cedarwood oil. All figures, except Pl. 3, fig. 21, are of the nerves of the hind-limb of the rabbit.

Abbreviations used in the plates

ax.	axone	*mac.n.*	macrophage nucleus
ax.e.	axone end-bulb	*mit.*	mitosis
ax.r.	axone remains	*myel.*	myelin sheath
c.	collagen	*my.r.*	myelin remains
e.	epineurium	*nlm.*	neurilemma
end.	endoneurium	*no.*	node
end.n.	endoneural nucleus	*nuc.*	nucleus
f.	fibroblast	*p.*	perineurium
i.	inclusion in Schwann cytoplasm	*S.b.*	bulb of cytoplasm at the end of a Schwann cell
inc.	Schmidt-Lantermann incisure	*S.c.*	Schwann cytoplasm
mac.	macrophage	*S.n.*	Schwann nucleus
mac.c.	macrophage cytoplasm	*S.t.*	Schwann tube

PLATE 1

All photographs on this plate are to the same magnification as indicated.

Fig. 1. Normal nerve fibres in longitudinal section, showing the relations of the Schwann nucleus and the cytoplasm around it with the myelin sheath and neurilemma. A node of Ranvier and incisures of Schmidt-Lantermann are also visible. Flemming, methyl benzoate-celloidin-paraffin, Masson.

Fig. 2. Normal nerve fibre showing the relations of the neurilemma at a node. Zenker, methyl benzoate-celloidin-paraffin, Masson.

Fig. 3. The extent of the Schwann cytoplasm within the Schwann tube after 6 days' degeneration. Picric-saline, Masson.

Fig. 4. Schwann cell after 15 days' degeneration. There are two large nucleoli, and the cytoplasm of the cell appears as a network over the myelin at the upper end of the figure. Flemming, Masson.

Fig. 5. Normal nerve fibres, showing the relations of Schwann nucleus, Schwann cytoplasm, myelin sheath and neurilemma. In both fibres in which a Schwann nucleus is visible the appearances suggest that at this level at least the Schwann cytoplasm extends completely around the outer surface of the myelin. Flemming, Masson.

Fig. 6. As fig. 5. Flemming, methyl benzoate-celloidin-paraffin, Masson.

PLATE 2

Fig. 7. A normal nerve fibre showing how endoneurium and neurilemma can be recognized near a node. Zenker, Masson.

Fig. 8. Schwann tubes in a peripheral stump after 25 days' degeneration. Around the macrophages the tubes are of normal diameter, but elsewhere they are collapsing. A Schwann nucleus is insinuating itself between the cytoplasm of the larger macrophage and the tube wall. Alcohol-formol-acetic, Masson.

Fig. 9. A peripheral stump after 15 days' degeneration. The myelin sheaths are blackened with osmium tetroxide: it can be seen that the myelin is being removed first from the outer surface of the sheath. Flemming, Masson.

Fig. 10. Peripheral stump 1 cm. below a suture made 25 days before. At the top of the figure a mass of Schwann cytoplasm, with an inclusion, is dammed up against a macrophage in a tube of which the outlines are only faintly indicated. Regenerating fibres can be seen growing down the inner surface of the tube walls. Alcohol-formol-acetic, Bodian.

Fig. 11. A peripheral stump after 15 days' degeneration. The neurilemmal walls of the Schwann tubes are clearly shown. To the left a tube which has collapsed except around the macrophages is seen, and Schwann nuclei are obstructed on either side of the upper macrophage. In the right-hand tubes myelin removal is less advanced. Flemming, Masson.

Fig. 12. A peripheral stump after 15 days' degeneration, showing to the left the Schwann cytoplasm extending over the inner surface of a tube wall, and surrounding unstained myelin remains. To the right are osmiophil remains. Flemming, Masson.

Fig. 13. A Schwann tube after 16 days' degeneration. At the lower end of the figure it is of normal diameter around a macrophage, but the rest is collapsed, and contains two Schwann nuclei, one pressed against the macrophage, and several strands of Schwann cytoplasm running together inside it. Alcohol-formol-acetic, Bodian.

Fig. 14. Two Schwann cells within a single tube, of which the walls are not stained. The fibrous cytoplasm of each cell extends past the nucleus of the other. Alcohol-formol-acetic, Bodian.

PLATE 3

Fig. 15. A peripheral stump degenerated for 353 days, showing the Schwann tubes full of darkly stained Schwann cytoplasm except at a point in the middle of the figure, where it is obstructed by a small remaining macrophage island. The stump was isolated by a second operation 10 days before fixation. None of the structures shown are nerve fibres. Formol, Bodian.

Fig. 16. Columns of Schwann cells, one of them showing mitosis, grown out from the end of a peripheral stump cut 15 days before, after having previously been degenerated for 53 days. A nerve fibre threads its way along one of the columns. Formol acetic saline, Mallory.

Fig. 17. A column of Schwann cells grown out from a peripheral stump during 15 days after section. The dense bands of Schwann cells lie amongst connective tissue containing fibroblasts. Zenker, Masson.

Fig. 18. Columns of Schwann cells from same slide as fig. 16, showing by the cracks between them that they are not a homogeneous syncytium. A mitotic figure in metaphase is seen to the left. Alcohol-formol-acetic, Mallory.

Fig. 19. The outgrowth from a peripheral stump 30 days after section. The stump is at the bottom of the figure, and the darkly stained columns of Schwann cells are seen growing out from it into fibrous tissue, much of the latter apparently arising from the epineurium. Formol-acetic-saline, Mallory.

Fig. 20. A Schwann tube after 25 days degeneration. A group of Schwann nuclei is lying on either side of a macrophage within the tube; further from the macrophage there are few nuclei. Alcohol-formol-acetic, Bodian.

Fig. 21. A human nerve degenerated for 26 days, showing a Schwann nucleus lying between two macrophages and deformed by them. The outlines of the macrophages and tube walls are only very lightly stained. Formol, Bodian.

Fig. 22. Schwann nuclei after 82 days' degeneration, two of them pressing against opposite surfaces of a macrophage. Alcohol-formol-acetic, Bodian.

Fig. 23. A peripheral stump after 15 days' degeneration, showing a Schwann tube in which two clearly distinct masses of Schwann protoplasm, each with its own nucleus, are lying alongside each other. Flemming, Masson.

PLATE 4

Fig. 24. Schwann cells in a peripheral stump degenerated for 83 days and containing no nerve fibres. A single cell runs the length of the figure to the right of the middle line. The Schwann tube is very lightly stained. Formol, Protargol method (see p. 66).

Fig. 25. A Schwann cell with an extremely elongated nucleus from a stump degenerated for 335 days. Both nucleus and cytoplasm have been compressed from the condition seen in fig. 24 by the contraction of the Schwann tube. Formol, Bodian.

Fig. 26. Schwann cells from the same material as fig. 24, showing nuclei in contact with macrophages, and the elongated axone-like appearance of the Schwann cytoplasm. Formol, Protargol method.

Fig. 27. Two Schwann cells lying together in a single (unstained) Schwann tube after 85 days' degeneration. The fibrous process of one cell is alongside the nucleus of the other. Alcohol-formol-acetic, Bodian.

Fig. 28. Schwann cell after 335 days' degeneration. Its cytoplasm shows longitudinal fibrils, and its nucleus has a median transverse constriction. Formol, Bodian.

Fig. 29. Schwann cell within a Schwann tube containing a macrophage after 83 days' degeneration. Formol, Protargol method.

Fig. 30. Schwann cells and axones in a peripheral stump degenerated for 85 days. Alcohol-formol-acetic, Bodian.

Fig. 31. Same material as fig. 30 but from a region into which a few nerve fibres have penetrated. The two Schwann nuclei indicated appear to lie within the same elongated protoplasmic band. Although this is fibrous in appearance it can be distinguished from the more darkly stained axone above it. Alcohol-formol-acetic, Bodian.

Fig. 32. Transverse section of the posterior tibial nerve cut and sutured 3 months previously. Zenker, Mallory.

Fig. 33. Transverse section of the posterior tibial nerve of the opposite limb to that shown in fig. 32. On this side the nerve was first degenerated for 16 months and then re-innervated for 3 months. In spite of the re-innervation the endoneurium is still very thickened. Zenker, Mallory.

Plate 5

All figures on this plate are to the same scale as indicated.

Figs. 34–39. Schwann tubes in long degenerated peripheral stumps, showing the varying appearances which result from shrinkage during fixation and embedding. All are stained with Masson's stain.

Figs. 34–37. From the same animal in which the nerve was degenerated for 83 days. Figs. 34–36 are of material fixed in Flemming, while that in fig. 37 was fixed in Bodian's alcohol-formol-acetic mixture. In all of them the neurilemma is distinct, as the wall of the Schwann tube. In fig. 35 a Schwann tube (*S.t.*2) containing a Schwann nucleus is filled with Schwann cytoplasm showing almost homogeneous appearance. In another tube (*S.t.*3) two Schwann nuclei with their cytoplasms lie side by side. In figs. 34 and 37 some of the tubes show the shrinkage of the Schwann cytoplasm which gives it the appearance, either of being extremely vacuolated (*S.t.*1) or of lying in strands around the inner surface of the neurilemma, leaving an apparently empty space in the middle of the tube (*S.t.*5, *S.t.*6). In fig. 36 the Schwann cytoplasm is quite well fixed, but shows irregularities as in (*S.t.*4).

Fig. 38. Stump degenerated for a longer time (241 days), showing the Schwann cytoplasm as a homogeneous mass filling all the space within the neurilemma. A suggestion of division between two separate cytoplasmic masses can be seen (*S.t.*7). Flemming, Masson.

Fig. 39. Still older stump (514 days) fixed in alcohol-formol-acetic, showing very poor fixation of the Schwann cytoplasm, which appears as a mass of separate threads. The increase in endoneural collagen is well shown. Masson.

Plate 6

Figs. 40–42. Terminal enlargements of the cytoplasm of Schwann cells in a nerve degenerated for 335 days (formol, Bodian). In fig. 40 two strands of cytoplasm lie together in a Schwann tube, and one of them terminates in a large club-shaped structure. In fig. 41 a similar but more elongated bulb lies against a Schwann nucleus, which itself is connected with a thin strand of cytoplasm. Another similar structure is seen in fig. 42.

Fig. 43. Several strands of Schwann cytoplasm are shown in a stump degenerated for 85 days. In the middle of the figure a large mass of Schwann cytoplasm can be seen. Alcohol-formol-acetic, Bodian.

Fig. 44. The outgrowth of axones and Schwann cells from the central end of the peripheral stump from animal 644 (p. 75) degenerated for 237 days and then cut again. Alcohol-formol-acetic, Bodian.

Fig. 45. Transverse section of peripheral stump degenerated for 33 days. The Schwann cells and neurilemma are not well demonstrated, but the pattern of the Schwann tubes, maintained by the neurilemma and endoneurium, is shown. Bouin, Masson.

Figs. 46, 47. Transverse section taken a few millimetres from the central end of a peripheral stump degenerated for 310 days. The neurilemma is well preserved, but the space between it and the darkly stained bands of Schwann cytoplasm is filled with material, presumably collagen, which is stained green. Alcohol-formol-acetic, Bodian, Masson.

Plate 7

Figs. 48–50, 52 and 53. Transverse sections of peripheral stumps taken 10 mm. below primary sutures made 25 days previously. They may be compared with fig. 51 which is of a peripheral stump degenerated for the same period but not re-innervated. All are fixed in alcohol-formol-acetic, stained by Bodian's method, counterstained Masson. In all the figures the Schwann cytoplasm is distributed around the inner surface of the walls; the lumen is occupied by macrophages and axone and myelin remains. The regenerating axones nearly all lie at the edges of the tubes and have presumably grown down between the Schwann cytoplasm (or macrophage) and the neurilemma. A few fibres (*ax.*1)

seem to lie within the Schwann protoplasm but the shrinkage makes it hard to decide the exact relations. Some axones are flattened between a macrophage and the tube wall (*ax.*2).

Figs. 54, 55. The same preparation taken at different focus to show numerous axones running in the wall of a Schwann tube, around a macrophage. In fig. 55 a small tube at the top of the figure also shows two fibres close to its wall.

PLATE 8

Fig. 56. An uninnervated peripheral stump after 25 days' degeneration, fixed in alcohol-formol-acetic and stained by Bodian's method without counterstain. Only the nuclei have taken up the silver and the outlines of the Schwann tubes are, therefore, barely visible.

Figs. 57, 58. Re-innervated Schwann tubes from sections 10 mm. below primary sutures made 25 days before. Alcohol-formol-acetic fixation, Bodian's stain, no counterstain. The section is directly comparable with the uninnervated stump in fig. 56, although the nuclei in this preparation have only slightly taken up the silver. At least thirteen regenerating axones lie around the inner surface of the walls of the large Schwann tube (*S.t.*1).

Fig. 59. Longitudinal section to show fibres growing into a homograft placed 25 days earlier. A single axone growing down a Schwann tube can be traced along the length of the figure from the top at the right of the mid-line. Peripherally, at the bottom of the figure, the axone is obstructed and sends out a smaller lateral branch shown at a higher magnification in fig. 61. Alcohol-formol-acetic, Bodian, Masson.

Fig. 60. Higher power view of a part of the field shown in fig. 59 showing the relations of a regenerating axone with the cytoplasm and nucleus of a Schwann cell.

Fig. 61. Higher power view of a part of the field shown in fig. 59, showing the blunted end of the axone, presumably caused by an obstruction, and the small lateral branch it is sending out within the Schwann tube. At this point it leaves the surface of one Schwann cell (*S.c.a.*) and becomes attached to another (*S.c.b.*), whose nucleus is somewhat abnormal.

Fig. 62. Longitudinal section of a peripheral stump 20 mm. below a primary suture made 25 days before. A single axone can be followed along a Schwann tube from the top of the figure at the right of the mid-line. It runs close to the surface of the Schwann cells. Alcohol-formol-acetic, Bodian, Masson.

PLATE 9

Fig. 63. Schwann tubes, one of them in the process of re-innervation, 10 mm. below a secondary suture made 15 days previously, the nerve having been predegenerated for 53 days. Separation between the parallel bands of Schwann cytoplasm can be seen within the tubes. Alcohol-formol-acetic, Bodian, Mallory.

Fig. 64. A stump degenerated for 150 days into which some fibres have penetrated and become medullated. In some cases there is more than one fibre within a single tube. The black points in the myelin sheaths are the result of silver impregnation after Zenker fixation. Zenker, Bodian, Masson.

Fig. 65. Peripheral stump immediately below primary suture made 82 days before. Several axones of considerable diameter are seen, and a few finer ones, one of which shows an end-bulb at a point at which it is obstructed by a nucleus. Alcohol-formol-acetic, Bodian.

Fig. 66. Transverse section 10 mm. below a primary suture made 107 days before. The Schwann tubes contain some large myelinated axones (*ax.*1) but there are also still many small axones around the inner walls of the tubes (e.g. *ax.*2). Alcohol-formol-acetic, Bodian.

Fig. 67. Longitudinal section of the suture line of a secondary suture 25 days old made after a delay of 38 days. A good union has been made between the stumps. Note that Schwann cells growing out from peripheral stumps collide in some places with fibres growing from the central stump. In figs. 67–70 the central stump is to the left. Alcohol-formol-acetic, Bodian, Mallory.

Fig. 68. Longitudinal section of a secondary suture 25 days old made after a delay of 150 days. There is a sharp demarcation between the stumps, and the union is thus less good than in fig. 67. Alcohol-formol-acetic, Bodian.

Fig. 69. Longitudinal section of a secondary suture 91 days old made after a delay of 365 days. Even though 3 months have elapsed after the suture there is still a clear distinction between the stumps, and although apposition is close, union has not been good. Alcohol-formol-acetic, Bodian, Masson.

Fig. 70. Longitudinal section of a secondary suture 97 days old made after a delay of 365 days. The union is rather better than in fig. 69, but there is much criss-crossing of axones at the suture line. Alcohol formol-acetic, Bodian, Mallory.

Fig. 71. Schwann outgrowth produced during 15 days after transection of the end of a peripheral stump that had previously been degenerated for 53 days. The stump is to the left, its end in the middle, and the whole of the right half of the picture thus shows new outgrowth. Alcohol-formol-acetic, Masson.

PLATE 10

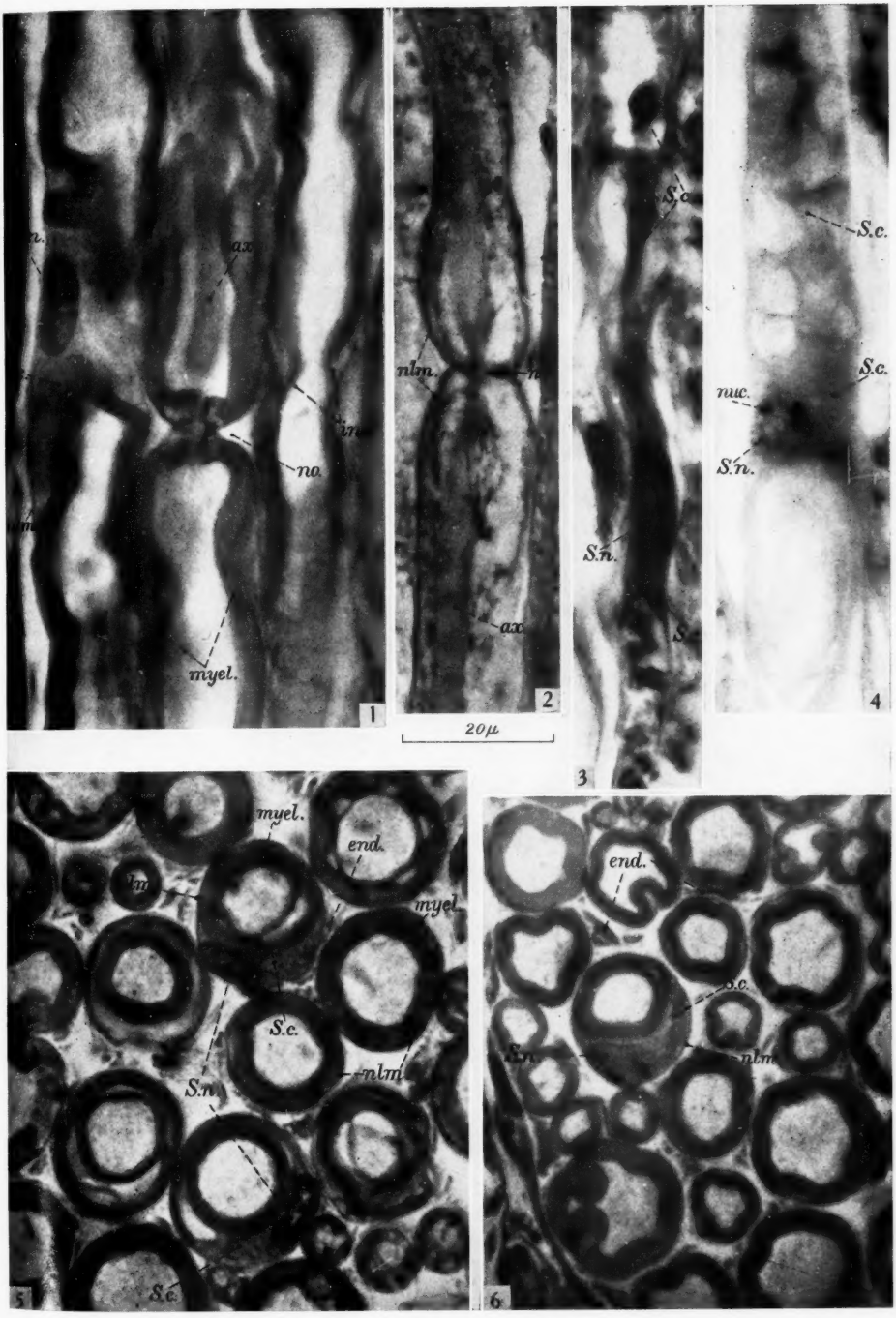
Fig. 72. A peripheral stump degenerated for 514 days. Section taken 85 mm. below secondary suture made 25 days previously. Nerve fibres have not yet reached this point. Alcohol-formol-acetic, Bodian, Masson.

Fig. 73. Same stump as fig. 72 but section taken 10 mm. below the suture. Nerve fibres are present. They are larger and fewer than after primary suture (cf. figs. 48–50, 57, 58) and the endoneurium is much thickened. Alcohol-formol-acetic, Bodian, Masson.

Fig. 74. Section 10 mm. below a secondary suture made 15 days earlier into a stump which had been allowed to degenerate for 53 days. The Schwann tubes are less shrunken than in figs. 72 and 73, but the fibres growing into them are larger than after primary suture. Alcohol-formol-acetic, Bodian, Masson.

Fig. 75. Section 10 mm. below a secondary suture made 97 days earlier after previous degeneration for 365 days. Some of the axones are now large and undoubtedly lie within the Schwann protoplasm. Alcohol-formol-acetic, Bodian, Masson.

Figs. 76, 77. Comparable transverse sections at the same magnification taken at the same distance below primary and secondary sutures respectively, made 103 days previously in the opposite limbs of one animal. The secondary suture (fig. 77) was made after a delay of 483 days, and the myelinated fibres are smaller in diameter, fewer, and more patchy in distribution than on the control side (fig. 76). Flemming, Weigert.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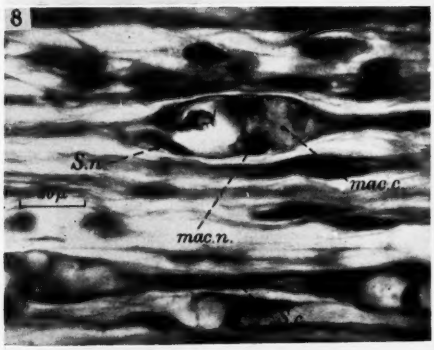
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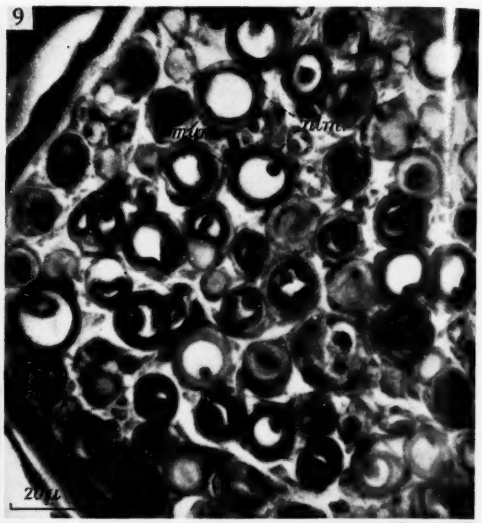
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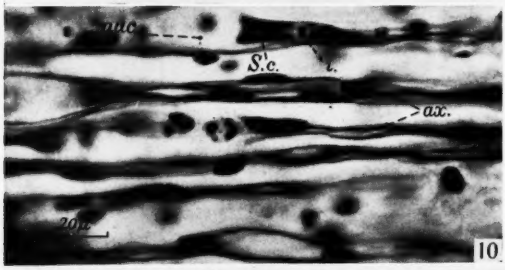
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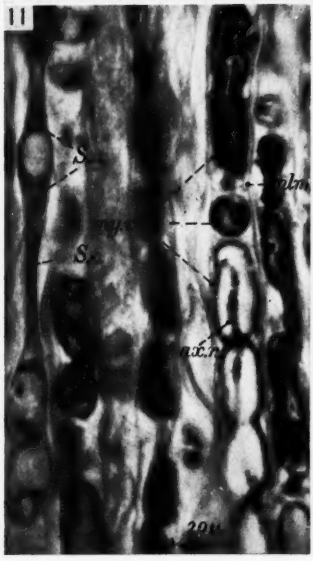
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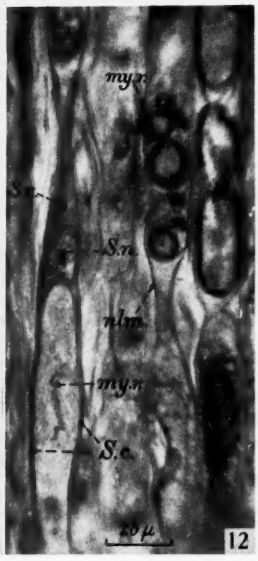
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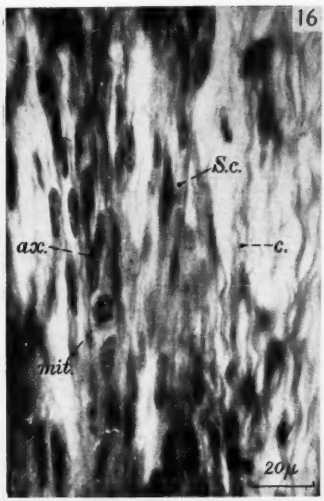
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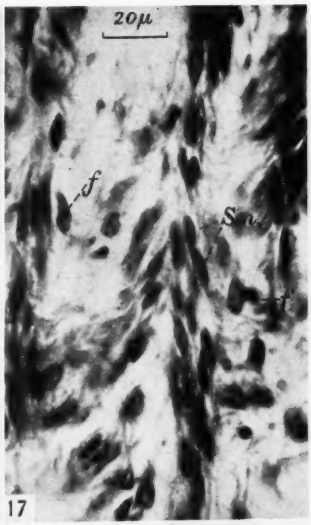
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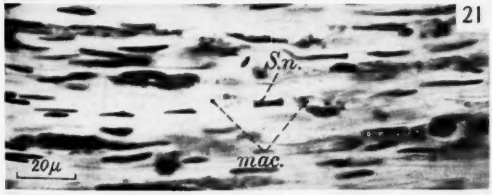
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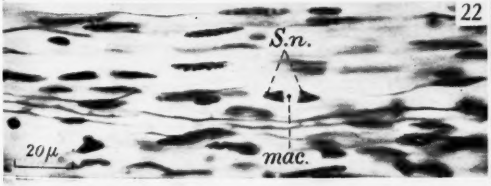
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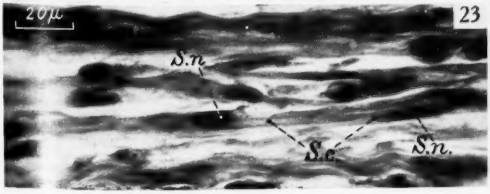
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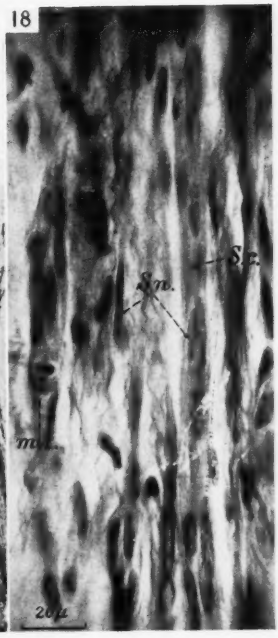
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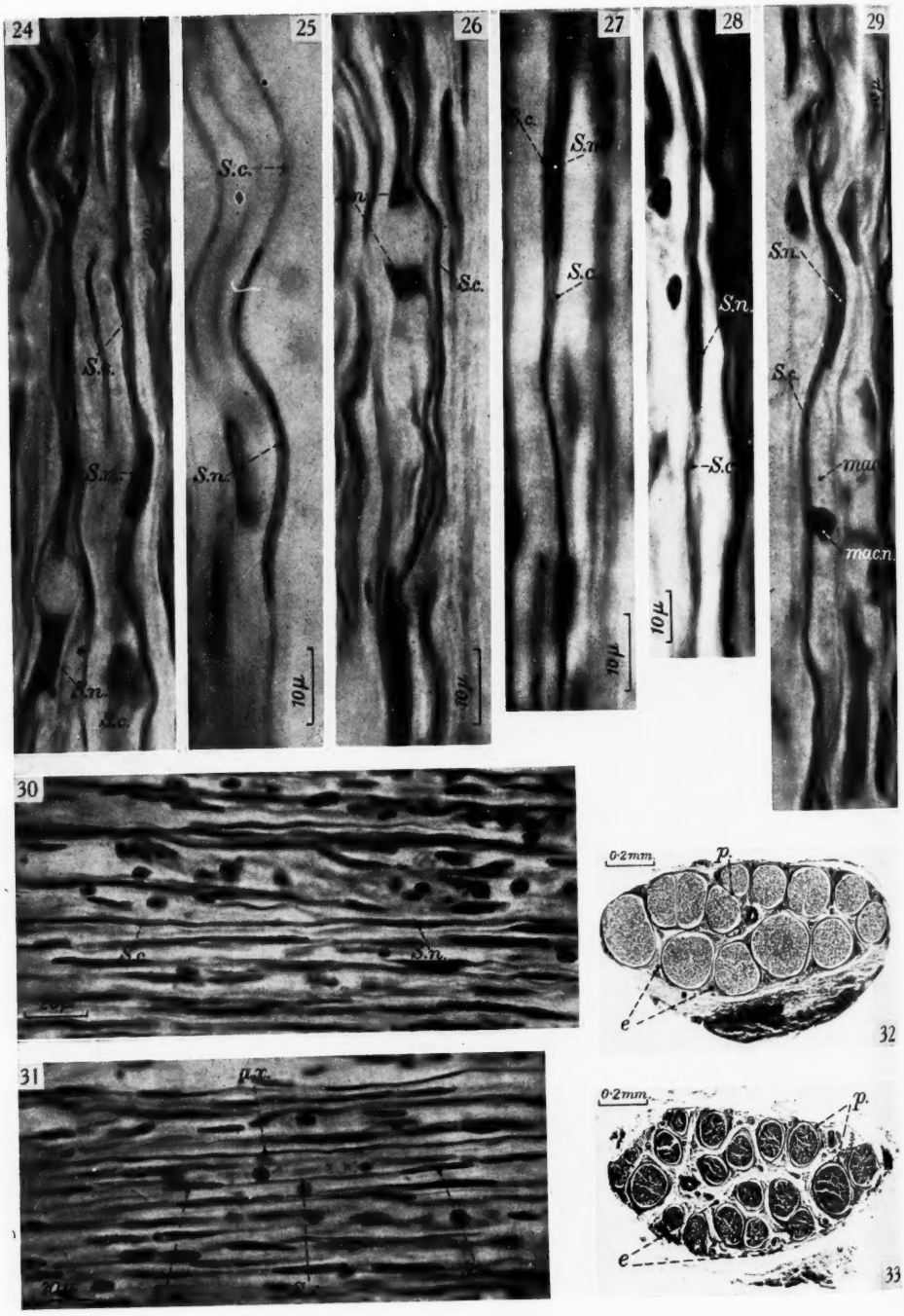
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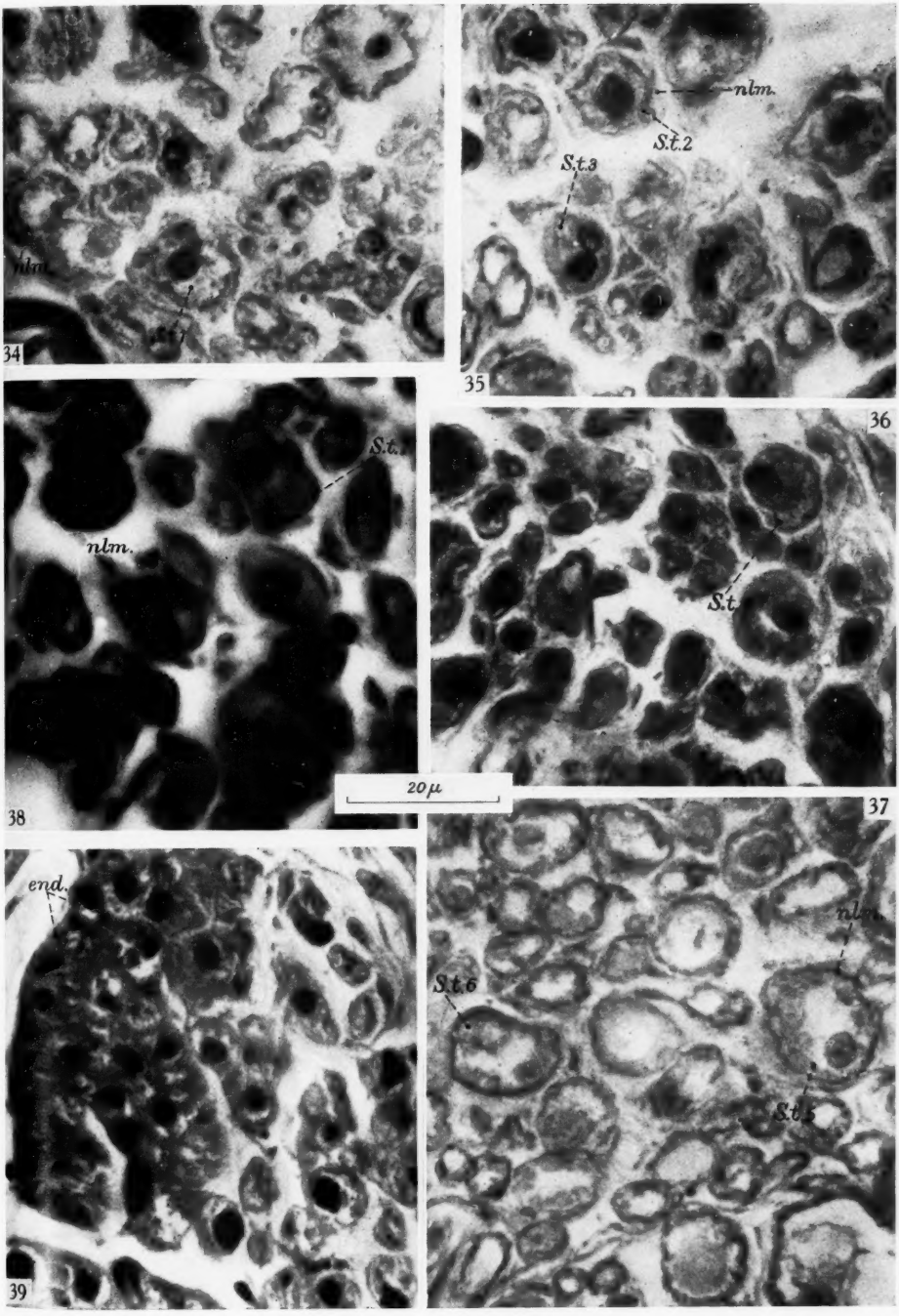
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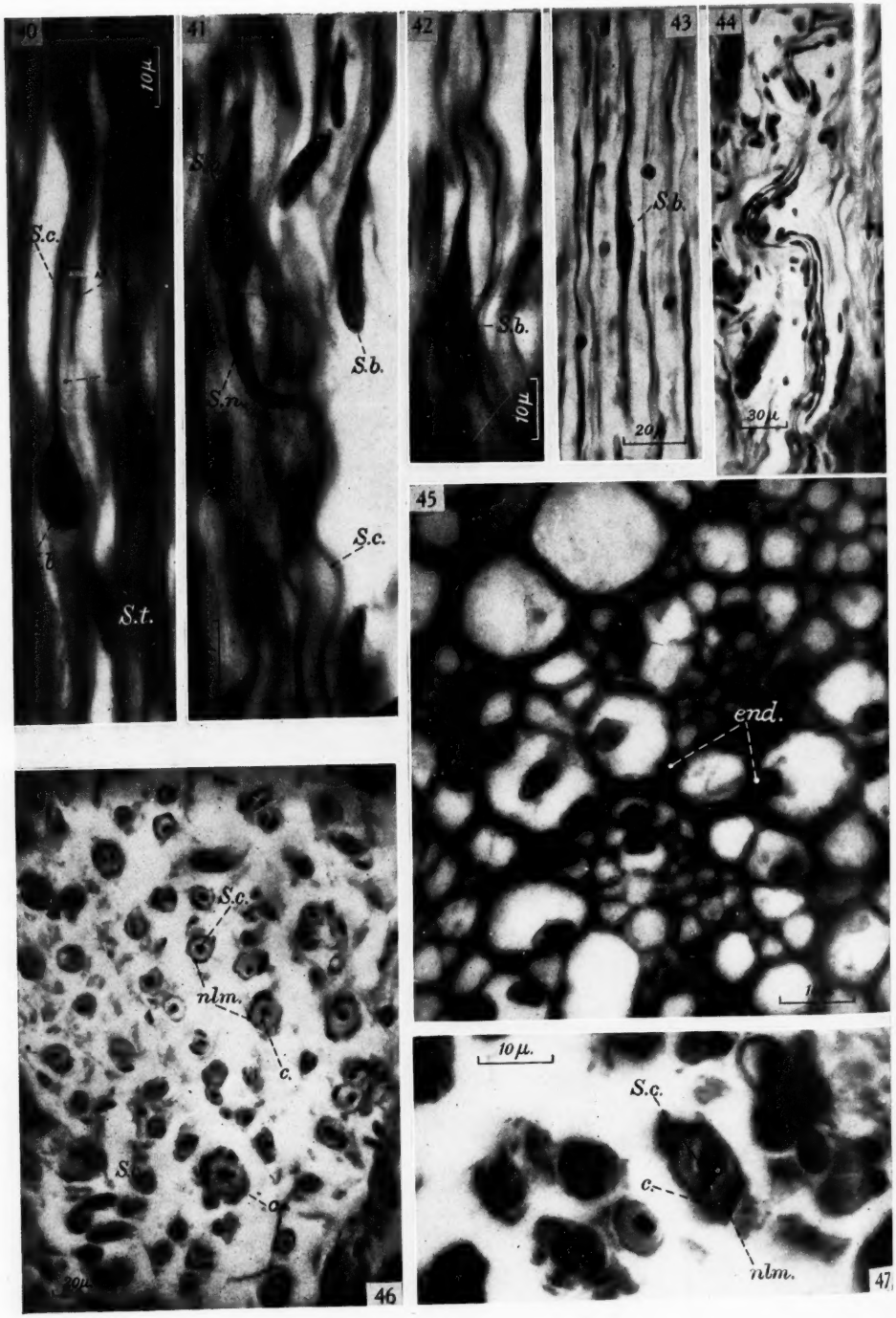

HOLMES AND YOUNG—NERVE REGENERATION

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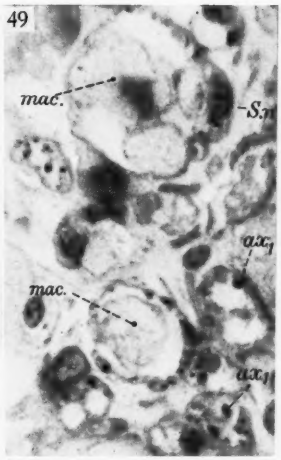
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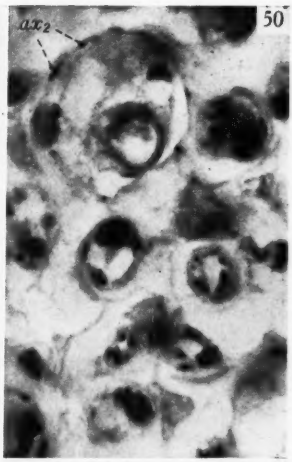
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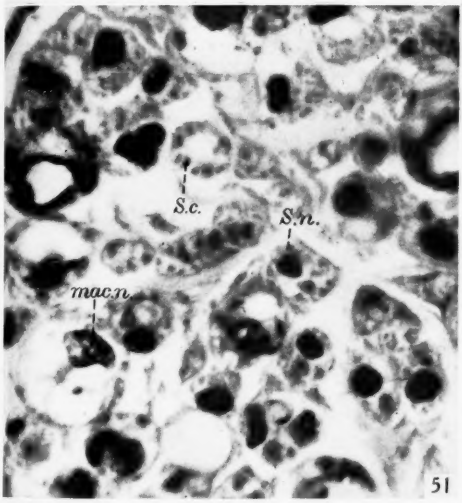
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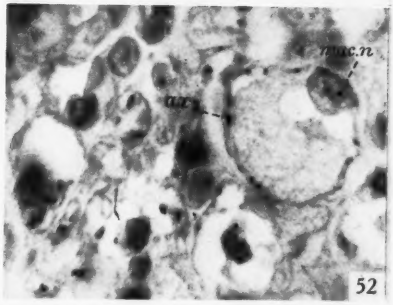
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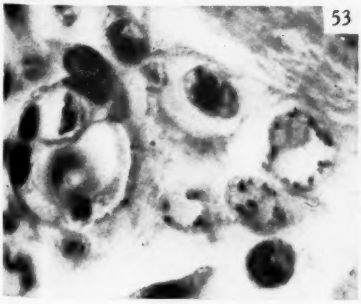
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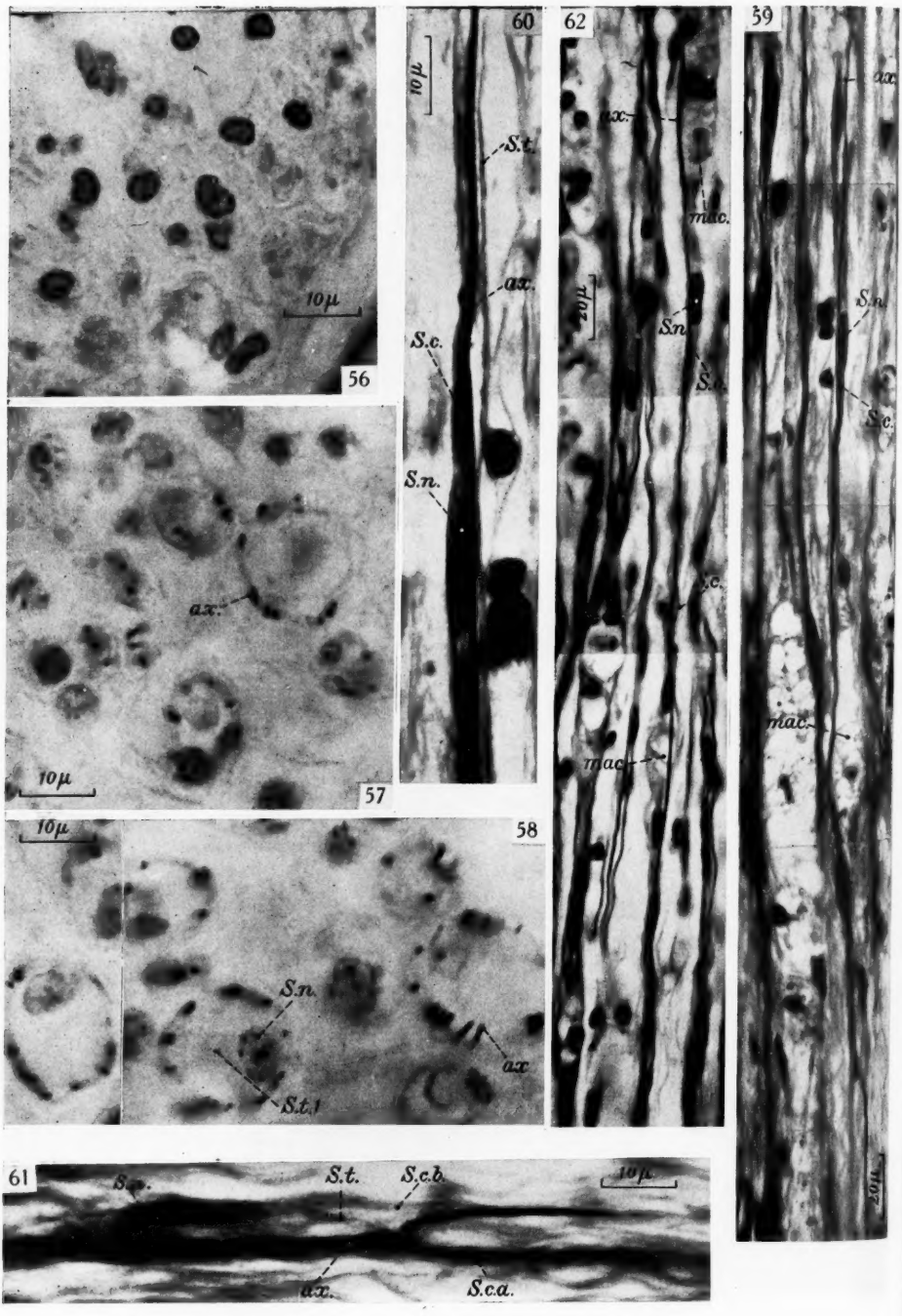
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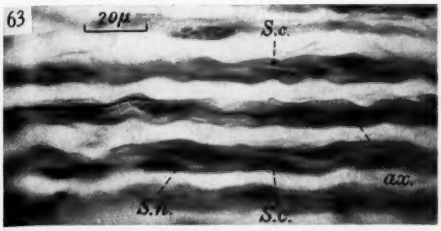
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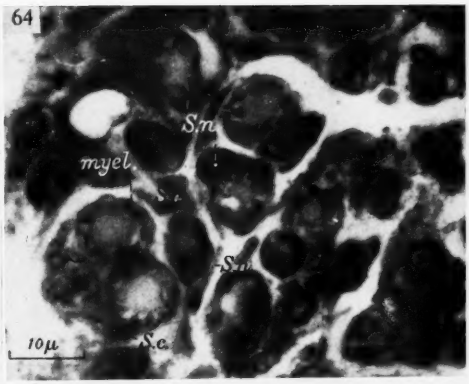
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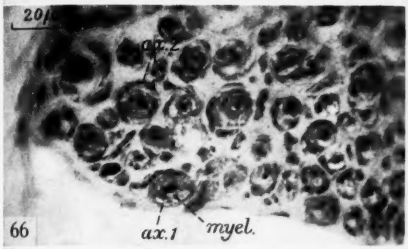
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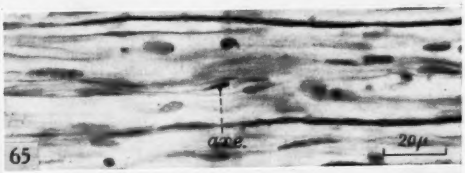
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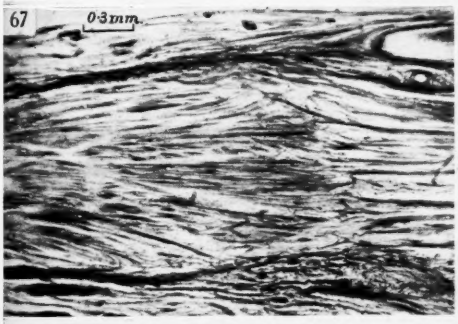
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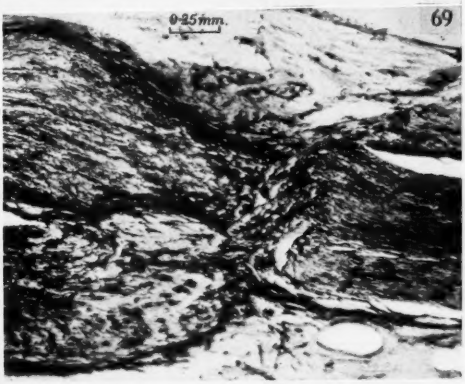
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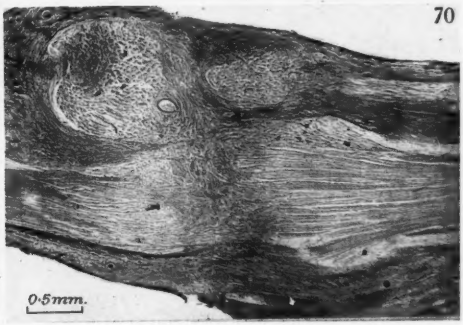
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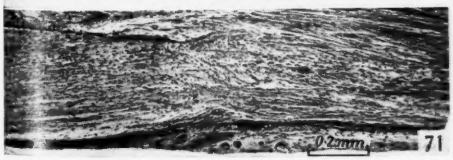
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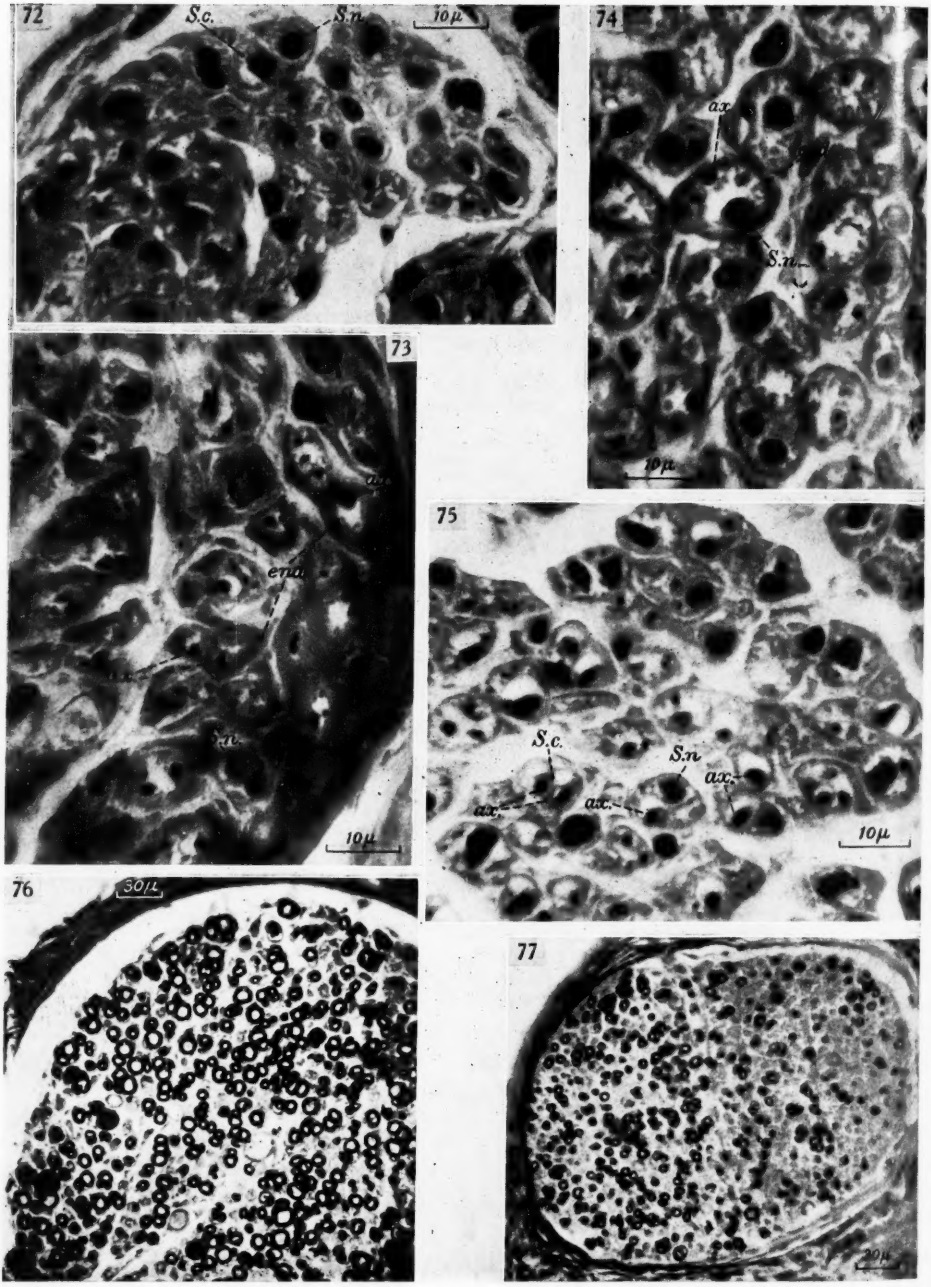
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A RADIOLOGICAL STUDY OF DEGLUTITION

By A. S. JOHNSTONE, *Department of Radiology, University of Leeds*

This paper is chiefly concerned with the action of the epiglottis. Prior to the publication of Barclay's work (1930, 1936) the generally accepted view was that the epiglottis turned over during swallowing, acting both as a chute for food and a guard for the larynx. Barclay, after an exhaustive radiological study, supported the theory that the epiglottis was a vestigial remnant, which served no useful purpose and stood erect during swallowing 'like a rock under a waterfall'. This theory has never appealed to the present writer, especially as he was once fortunate enough to see the epiglottis turn over in a patient with a lateral pharyngotomy. It should be noted, however, that this observation is in direct opposition to that made by Anderson Stuart (Barclay, 1936) on a similar case. Recently an article by Welin (1939) described some cases of dysphagia due to the slow return of the epiglottis to the erect position. In a general discussion he gave the impression that the theory upheld by Barclay and Negus (1929) had gained little support in Europe. It came therefore as a surprise to find, during a recent perusal of the British literature, that the common teaching of anatomists and physiologists is that the epiglottis remains upright during deglutition.

Radiologists have great opportunities to study this question and, if the pharynx of each case for barium-meal examination is carefully screened, considerable variations in the speed of swallowing will be found. In the majority the movements of the epiglottis are too quick for the eye to follow, but in some the process is slower and the whole range is clearly discerned. It is these cases which leave absolutely no doubt in the mind of the author and his colleagues that the epiglottis does turn over. The return to the erect position is most impressive when it moves slowly and deliberately up the posterior pharyngeal wall and then slips off the wall to flick suddenly back to its normal position. Without some form of cinematograph the complete sequence of movement cannot be analysed but, with careful timing, radiographs have been obtained to confirm the following observations.

Observations made during swallowing. After taking the bolus the mouth is closed and the tip of the tongue rises to the palate, allowing the food to fall into the hollow of the tongue. If fluid opaque medium is used, it will be seen to flow back into the valleculae. To complete the voluntary stage the tongue, hyoid bone and larynx are forcibly and suddenly raised, so that the tongue is pressed against the palate and the barium is projected through the fauces into the pharynx. At the same time the upper nasal passages are cut off from the oral pharynx anteriorly by the approximation of the posterior faucial pillars and uvula, and posteriorly by the superior pharyngeal constrictors forming Passavant's ridge. The approximation of the anterior faucial pillars and the tongue cut off the return to the mouth. The pharynx seems to be drawn up over the descending bolus, and finally the base of the tongue bulges downwards and backwards, meeting the posterior pharyngeal wall which becomes thickened by contraction of the middle group of constrictors; together these act as a piston, giving a real thrust downwards to the bolus.

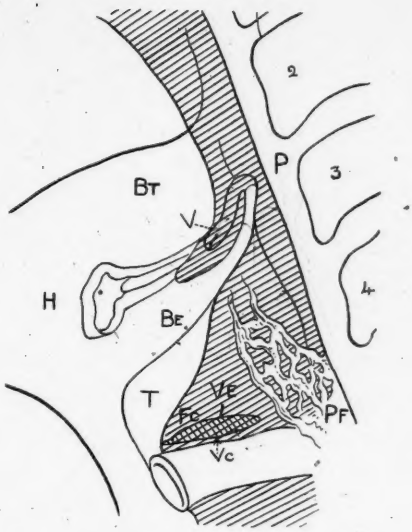
To protect the larynx, the vestibule must be closed, and this is done by a combination of several factors. The whole larynx is drawn up, first by the hyoid bone which also moves forwards, and then by a marked elevation of the thyroid cartilage. Sometimes the upper border of the latter passes well behind the hyoid bone. This causes the pad of fat at the base of the epiglottis to bulge backwards, pushing out the tubercle so that it readily meets the closed arytenoids as they move upwards and forwards. The base of the tongue now rolls the upper part of the epiglottis backwards with the arytenoids acting as a fulcrum and, assisted by the contraction of the small epiglottic muscles, the tip turns over, covering the ostium like a lid. The bevelled margins direct the stream of food or fluid into each lateral channel leading to the pyriform fossa. The valleculae, opened out by the pivoting of the epiglottis, form a surprisingly large space. Although it seems that the weight of a large bolus would itself push over the epiglottis, there is adequate mechanical and muscular stress to do so for it occurs equally well when swallowing saliva or sips of fluid. At this stage the radiograph shows the vestibule to be devoid of air, but the margins of the vocal cords are sharply delineated by air in the larynx.

If several swallows are made without interruption for respiration, then the epiglottis remains overturned. When the act is completed the hyoid bone and thyroid cartilage drop back and the base of the epiglottis pivots round so that the tip is a little bent over as it slides up the posterior pharyngeal wall. The restoration is completed by the recoil of the elastic tissue. The laryngeal vestibule and pharynx open up and are clearly outlined by air shadows. As mentioned before, the slow deliberate movement seen in a number of normal people gives a most impressive picture, and it is almost possible to persuade oneself that there is a click as the epiglottis finally springs back to complete its return.

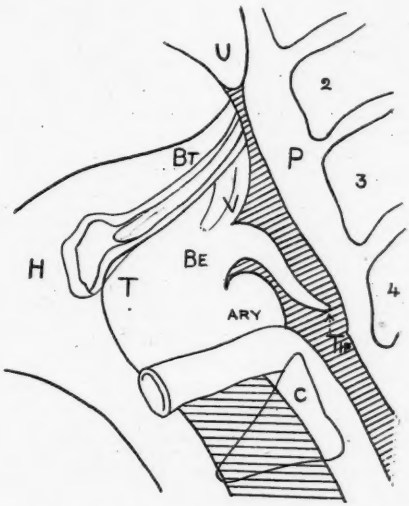
DISCUSSION

Barclay admits that considerable variations can occur and propulsive effort may be required, but he contends that the main factor in swallowing is negative pressure in the pharynx and upper oesophagus. This has not been confirmed by observations made during reconstruction operations following oesophagectomy. In fact, the force with which food is pushed down an artificial gullet is astonishing and could only be produced by considerable *vis a tergo*. Furthermore, there is no evidence of suction through a high oesophagostomy which would surely result from such negative pressure. Barclay denies any change in the posterior pharyngeal wall when he writes: 'there is no suggestion that the posterior pharyngeal wall comes forward at all or any other sign that indicates a constrictor action of its muscles.' There is no doubt, however, that there is considerable thickening of the posterior pharyngeal wall, presumably from contraction of the constrictors, and the way in which the middle group meets the base of the tongue to administer the final push is clearly illustrated in Text-fig. 2 and Pl. 1, figs. 2 and 4.

When Barclay is discussing the obliteration of the air shadow in the vestibule of the larynx he says: 'something comes from above and slips down between the hyoid and the epiglottis, forming a well rounded shadow across the top of the larynx and obliterating the whole larynx down to the false cords.' There is no doubt that this something which he fails to identify anatomically is the epiglottis, and the closure of the vestibule radiologically is seen by the absence of air after the sphincter-like contraction of the ostium and the elevation of the thyroid cartilage. These findings are



Text-fig. 1. Outline drawing made from the radiograph in Pl. 1, fig. 1, showing the normal resting phase of the epiglottis and related structures. *Bt.* base of tongue; *Be.* base of epiglottis and position of pad of fat; *P.* post-pharyngeal wall; *V.* valleculae; *H.* hyoid bone; *T.* upper border of thyroid cartilage; *Pf.* pyriform fossa; *Ve.* vestibule of larynx; *Fc.* false vocal cords; *Vc.* vocal cords.



Text-fig. 2. Outline drawing made from the radiograph in Pl. 1, fig. 2, showing the changes which occur just as the last part of the bolus leaves the pharynx. *C.* cricoid cartilage; *Ary.* arytenoid cartilages; *U.* uvula; *Tip.* tip of epiglottis. Other lettering as in Text-fig. 1.

well illustrated in Text-fig. 2, and Pl. 1, fig. 4, and it is clear that they are always present when the radiograph has been taken at the correct moment. Only three of Barclay's illustrations show this phase, and in two it is possible to trace the outline of an overturned epiglottis.

As a variation from the normal, the writer has seen two cases similar to those described by Welin, in which the return of the epiglottis was so slow as to cause a sensation of something sticking in the throat. These occurred in elderly people and the condition is probably caused by senescence of the fibro-elastic cartilage. In another case cerebral metastases had caused some paresis of the pharyngeal musculature, for there was no co-ordinated effort in swallowing, and barium passed readily into the larynx while the epiglottis remained erect.

It is well recognized that after removal of the epiglottis there is no difficulty in swallowing, and this is used as an argument in favour of the upright theory, but it should be remembered that in a surgical removal the base of the epiglottis is not excised.

SUMMARY

The theory, strongly supported by Barclay, that the epiglottis remains erect during swallowing, is discussed, and evidence, substantiated by radiographs, is given to show that the epiglottis does turn over. An attempt is made to explain this movement as occurring secondarily to interacting forces between the rising larynx and the downward push of the base of the tongue, but assisted slightly by the small intrinsic muscles. Barclay's theory of negative pressure as the chief factor in swallowing is considered to have no foundation, and the final thrust administered by the base of the tongue and the posterior pharyngeal wall is clearly illustrated.

I should like to acknowledge with gratitude the help received from Prof. A. Durward and Prof. John Kirk.

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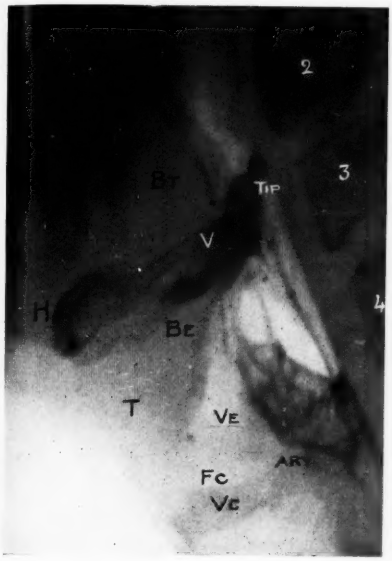
EXPLANATION OF PLATE 1

Figs. 1, 3. Radiographs showing the normal resting phase of the epiglottis and related structures. Note the erect epiglottis with traces of barium in the valleculae, the vestibule outlined by air, the space between the hyoid bone and the thyroid cartilage, and the width of the posterior pharyngeal wall.

Figs. 2, 4. Radiographs showing the changes which occur just as the last part of the bolus leaves the pharynx. Note the epiglottis overturned and clearly seen as the lateral channels are not flooded, the marked thickening of the posterior pharyngeal wall meeting the bulging base of the tongue, the wide space of the valleculae, the exclusion of air from the vestibule, and the slight impression made by the tip of the epiglottis in the posterior pharyngeal wall.

Key

U. uvula; *Bt.* base of tongue; *Be.* base of epiglottis and position of pad of fat; *P.* post-pharyngeal wall; *V.* valleculae; *H.* hyoid bone; *T.* upper border of thyroid cartilage; *Pf.* pyriform fossa; *C.* cricoid cartilage; *Ve.* vestibule of larynx; *Ary.* arytenoid cartilages; *Fc.* false cords; *Vc.* vocal cords; *Tip.* tip of epiglottis.



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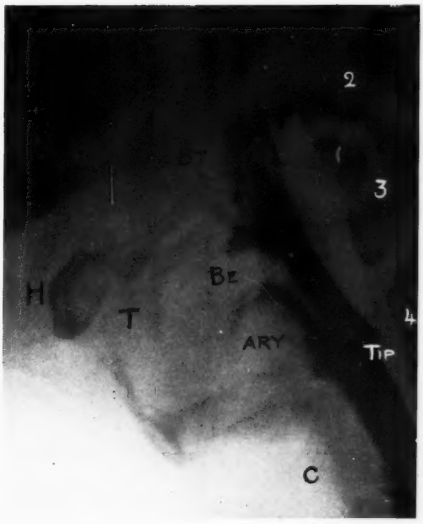
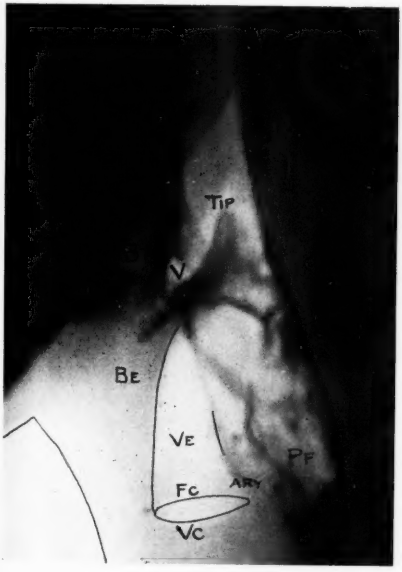


Fig. 2



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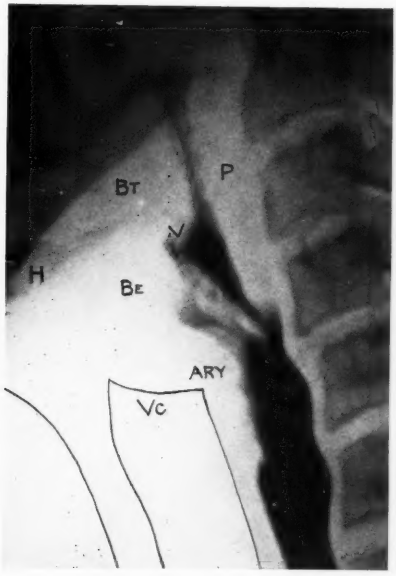

Fig. 4

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ANATOMICAL NOTES

A NOTE ON THE RENAL FASCIA

By C. P. MARTIN
Department of Anatomy, McGill University, Montreal

In a recent article Mitchell (1939) made some observations on the renal fascia which differ in certain respects from the descriptions found in most text-books. Cunningham's *Textbook of Anatomy*, 7th edition, for example, describes the renal fascia as consisting of two layers, an anterior and a posterior, and states that these two layers fuse together at the lateral and upper borders of the kidney where they become continuous with the fascia covering the transversus abdominis and diaphragm respectively. Below the kidney the two layers do not fuse but gradually fade out, while medially the posterior layer fuses with the fascia covering the psoas major and quadratus lumborum, and the anterior layer is continued towards the median plane in front of the renal vessels and aorta. Thus the anterior layer of the renal fascia on one side of the body is continuous with the corresponding layer of the other side. Gray's *Anatomy*, 23rd American edition, gives a very similar description; the 27th English edition, however, adds a footnote drawing attention to Southam's paper (1922–3). Buchanan's *Manual of Anatomy*, 6th edition, does not mention the renal fascia. Braus' *Anatomie des Menschen*, 1st edition, 1924, states that the renal fascia consists of prerenal and postrenal layers but makes no mention of the attachments or connexions of these layers. Poirier and Charpy, in the 3rd edition of the *Traité d'Anatomie Humaine*, state that the posterior layer is partially adherent to the aponeurosis of the diaphragm and to the psoas but apparently leaves these at the medial border of the kidney to join the anterior layer. The latter according to these authorities is weaker and less clearly defined than the posterior layer and blends medially with the fascial sheaths of the aorta, inferior vena cava, and their branches and tributaries.

Southam made extensive researches on the renal fascia both by dissections and by studying transverse and longitudinal sections through the kidney region. According to his account, at the medial border of the kidney the anterior and posterior layers of the renal fascia fuse together as far down as the hilum, but opposite and below the hilum they remain separate, the posterior layer fusing with the periosteum of the lumbar vertebrae, while the anterior loses itself in the connective tissue in front of the aorta and inferior vena cava. Mitchell studied the renal fascia by injecting radiologically opaque material into the perirenal space between the kidney and the fascia, and determined by X-rays and dissections the boundaries of the space occupied by this material and the directions in which it spread. He states that the posterior layer of the fascia blends medially with the fascia on the psoas major and that the anterior layer is attached to the bodies of the vertebrae and the intervertebral discs.

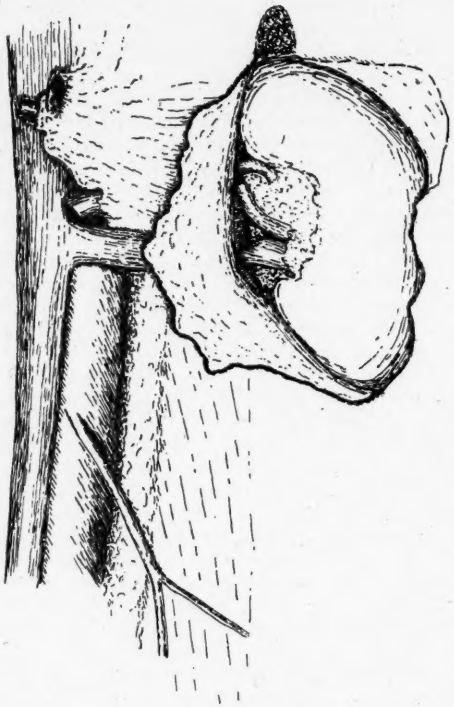
From the above accounts it is evident that there is a considerable divergence of opinion as to the exact arrangement of the fascia. There is general agreement that at the lateral border of the kidney the two layers join and fuse with the transversalis fascia; at the upper end of the kidney they likewise join and blend with the fascia on the diaphragm; while at the lower pole of the kidney they remain distinct and fade out as they are traced downwards. There is no agreement, however, regarding the arrangement or attachments of the layers at the medial border of the kidney. If the views of either Mitchell or Poirier and Charpy are correct, it is evident that the renal vessels must pierce the renal fascia; if, on the other hand, the descriptions given in Cunningham or Gray's *Anatomy* are correct, it is difficult to understand why an effusion into the perirenal space on one side of the body does not extend across into the corresponding space on the other side.

An effort was therefore made to determine which of these descriptions is the more correct. A search for the renal fascia was made in 32 subjects which were being dissected in the laboratory; and, in addition, a special dissection was carried out on another subject and transverse sections of the body at the kidney level were carefully studied. These investigations led to the conclusion that while all of the above descriptions of the renal fascia are in a sense correct they are incomplete.

First, from the bodies studied it is evident that a layer of fascia can be traced from the anterior aspect of one kidney across the midline to the anterior aspect of the other. This

layer is very thin in front of the aorta and its continuity across the midline can be traced superiorly only as high as the origin of the superior mesenteric artery. Above this level the anterior layer of fascia turns forwards to cover the mass of connective tissue surrounding the origins of the coeliac axis and superior mesenteric artery, and in which lie the coeliac and superior mesenteric autonomic plexuses.

If this anterior layer of fascia is incised in the midline and turned laterally, or, better still, if it is incised in front of the kidney and turned medially, it can be seen that at the medial border of the kidney the layer splits. As already mentioned, a superficial lamina passes across the midline of the body in front of the aorta and inferior vena cava, and a deeper stratum passes backwards around the medial border of the kidney. Text-fig. 1 is a drawing of such a dissection and the renal vessels can be seen piercing this deeper stratum of the fascia. By making a careful search for it, this deeper stratum could be demonstrated in almost all the well-preserved bodies examined.



Text-fig. 1. The anterior layer of the renal fascia has been turned medially and a deeper stratum can be seen passing back around the medial border of the kidney. This deeper stratum is pierced by the renal vessels.

With the object of demonstrating it in a more striking and convincing manner, a special dissection was made. In the body of a young adult female containing very little fat, a block of tissue was removed from the back extending from the 11th rib to the crest of the ilium, and from near the midline to a line about 6 inches from the median plane. The block extended deeply as far as the 12th rib and the quadratus lumborum muscle. The latter muscle was then carefully removed, thus exposing the posterior layer of the renal fascia from behind. The fascia was then incised and the kidney removed piecemeal through the incision. By this procedure the anterior and medial parts of the renal fascia were not disturbed. The renal vessels and ureter were cut at the hilum of the kidney.

Fig. 1

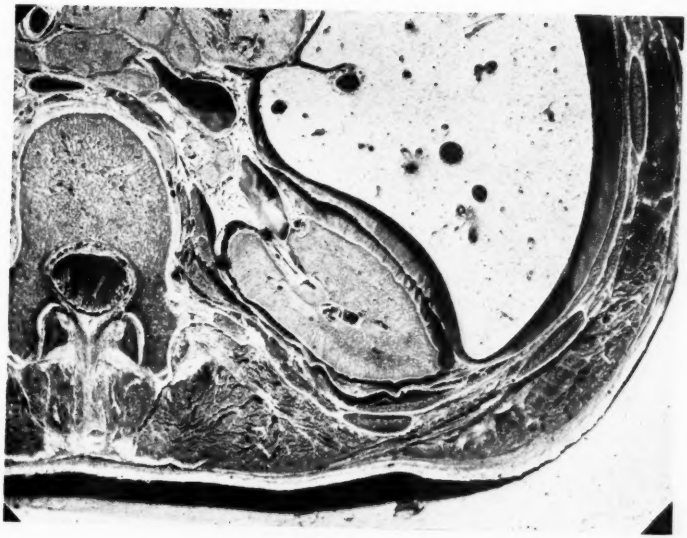


Fig. 2

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Pl. 1, fig. 1 is a photograph of the space from which the kidney was thus removed. It can be seen quite plainly that the renal vessels and ureter are passing through a layer of fascia and that the anterior and posterior layers of the renal fascia are in fact to some degree continuous with each other round the medial border of the kidney.

While making this dissection it was noticed that the posterior layer of fascia turns into the hilum of the kidney at the medial border of the organ, and becomes firmly attached to the posterior aspect of the pelvis of the ureter. This is clearly shown in Pl. 1, fig. 2, which depicts a transverse section through the body opposite the hilum of the right kidney. In the figure it can be seen that the posterior layer of fascia splits at the medial border of the kidney and the two resulting lamina diverge. As already mentioned, one turns into the hilum to blend with the posterior wall of the pelvis; the other is continued forwards and medially to blend with the fascia covering the psoas major muscle at its anterior edge. It will also be noticed that the post-renal fascia is likewise attached to the fascia of the psoas major at the lateral edge of that muscle. The continuity between the anterior and posterior layers of the renal fascia at the medial border of the kidney could not be demonstrated in transverse sections such as that figured here. The prerenal layer is easily separated from the kidney, but a short distance medial to the hilum it becomes firmly adherent to the front of the 'renal pedicle' consisting of the ureter, renal vessels and surrounding fascia and fat, and just in this region bands of fascia pass, as shown in Text-fig. 1, from the prerenal back to join the postrenal layer. This connecting layer of fascia, however, could not be demonstrated in these transverse sections through the hilum, possibly because it is broken up by the ureter and renal vessels.

Another observation made while carrying out the special dissection described above was that the perirenal veins lying in the perirenal fat between the renal fascia and the kidney are often of quite considerable size, sufficiently large, in fact, to have a definite 'nuisance value' during operations on the kidney. These veins are figured by Poirier and Charpy in the *Traité d'Anatomie Humaine*, but our own text-books generally ignore them. In this dissection a large perirenal vein ran over the dorsal aspect of the upper pole of the kidney and seemed to terminate in the renal vein. Its exact termination was not determined for it was damaged and had been partly removed during the piecemeal removal of the kidney.

Briefly, therefore, these investigations indicate that the anterior layer of renal fascia does continue across the midline of the body in front of the aorta and inferior vena cava to become continuous with a similar layer on the opposite side of the body, but this continuation in front of the great vessels is very thin and only extends as a distinct layer upwards as far as the origin of the superior mesenteric artery. The posterior layer of the renal fascia blends with the fascia covering the psoas major, especially at the medial and lateral edges of this muscle. But, in addition to these two layers, there is also a deeper stratum connecting the anterior and posterior layers around the medial border of the kidney, and this deeper stratum is pierced by the renal vessels and ureter. At the upper pole and lateral border of the kidney the anterior and posterior layers of the fascia fuse; at the lower pole they also fuse, but much more weakly than in these other locations. Incidentally, the perirenal veins may be sufficiently large to merit attention during operations on the kidney.

These findings agree generally with the results of Mitchell's injection experiments but they differ in detail from both his and Southam's description of the fascia.

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EXPLANATION OF PLATE 1

Fig. 1. 'The kidney bed' seen from behind. The kidney has been removed piecemeal without disturbing the fascia on its anterior and medial aspects. The renal vessels and ureter can be seen piercing the renal fascia. *A* is the 12th rib.

Fig. 2. Transverse section through the body opposite the hilum of the right kidney. The renal fascia can be seen distinctly. The posterior layer of the fascia splits at the medial border of the kidney. One stratum passes into the hilum of the kidney and the other passes forwards and medially to blend with the fascia at the anterior edge of psoas major.

A NOTE ON THE CLOSURE OF THE FORAMEN OVALE AND THE POST-NATAL CHANGES OF THE VENTRICLES IN THE HUMAN HEART

By J. A. KEEN, *Department of Anatomy, University of Cape Town*

In the recent literature on the foetal circulation are descriptions of many investigations made on mammals during life (Pohlman, 1909; Kellogg, 1928, 1930; Barclay, Barcroft, Barron & Franklin, 1941; Franklin, Barclay & Prichard, 1940; Windle & Becker, 1940), but less attention seems to have been given to the anatomical conditions in the full-term human foetal heart. Embryology text-books usually concentrate on the earlier stages in the development, and detailed descriptions of the changes around the foramen ovale at or soon after birth are not given. Experiments similar to those carried out on lower animals are not possible in the human subject, and so examination of the heart post-mortem is the only method of study available in man.

Closure of the foramen ovale. The material used in this investigation comprised hearts removed from the bodies of several full-term foetuses and young infants. The most suitable dissection to show the septum primum and septum secundum is one made from the back. Both atria are opened widely by the removal of their posterior walls, and, if one also cuts away the corresponding posterior half of the interatrial septum as far as the middle of the foramen ovale region, the valvular mechanism of the opening is clearly demonstrated.

Figs. 1–4 show the progressive changes involved in the closure of the foramen ovale in the human foetus and infant, from the completely open stage to the firm structural union of the two septa into one partition.

The heart seen in Fig. 1 came from a foetus which had been removed post-mortem, the mother having died of tetanus; she was stated to be 28 weeks' pregnant. The specimen shows the foramen ovale completely open.

The heart in Fig. 2 is that of a newborn male infant that had lived for a few hours. The septum primum is beginning to get applied to the septum secundum, but there is still an open channel.

The heart seen in Fig. 3 came from an infant which died, on the third day after birth, from a haemorrhagic condition. The septum primum lies against the septum secundum, but structural union has not yet taken place; the least pressure with a probe opens up the channel. The appearance is that of a functional closure at a stage before the flaps are anatomically united.

When the foramen ovale is at the stage of closure seen in Fig. 3, at or very soon after birth, the heart is particularly suitable for demonstrating the mechanism of the foramen by running fluids through the organ. The apex of the heart must be cut off and glass cannulae pushed into each ventricle, so as to make it easy to observe the flow of fluid either to the right or left side. The right and left atria are connected by tubing to fluid containers which can be raised or lowered, thus regulating the pressures. A weak solution of Prussian blue was found to be a very suitable liquid. If the solution is run into the right atrium, some is seen to flow out of the right ventricle and some out of the left ventricle. If the pressure of the fluid inflow into the right atrium is increased, a larger proportion of the liquid reaches the left side, showing that with the greater pressures the septum primum becomes pushed over to the left and the opening of the foramen ovale is made larger.

The effect of an increased pressure in the left atrium can also be shown quite easily. While the blue solution is running into the right atrium and is being distributed to the right and left sides, clear water is run into the left atrium at a slightly higher pressure. At once the blue colour begins to disappear from the left side and clear water comes out of the left ventricle, showing that the valve mechanism of the foramen ovale has come into operation. This simple experiment demonstrates the manner in which the functional closure of the foramen ovale presumably occurs at birth.

Fig. 4 shows an infant's heart with the adult circulation established. The foramen ovale is completely closed with firm structural union, but it is still possible to see the line of separation between the septum primum and the septum secundum.

Fig. 1. Dissection of the foramen ovale in the human foetal heart. Note the septum primum projecting well into the left atrium.

Fig. 2. Dissection of the foramen ovale in the human heart at birth. Note the septum primum still projecting into the left atrium, but much nearer the septum secundum than in Fig. 1.

Post-natal changes in the ventricles. Some observations were made on the relative thickness of the ventricular walls in these specimens. A piece of the heart near the apex was sliced off, at a level approximately three-fourths of the distance between the atrio-ventricular groove and the apex. The illustrations (Figs. 5–8) show the cut surface of the heart after the apical portion had been removed. The contrast between the relative sizes of the right and left ventricles at birth and in the early months after birth is very striking. In order to gain an estimate of the differences in size of the two ventricles at these ages, planimetric measurements of the cross sectional areas were made. In making the drawings for this purpose it is necessary to distinguish between the muscle of the ventricular walls and the papillary muscles occupying the cavity, and to exclude the latter from the measurements.

All the foetal hearts and those obtained from infants within a day or two of birth showed the appearance illustrated in Fig. 5. There were twelve specimens available which belonged to this age group and all showed the same features. The right and left ventricles took an

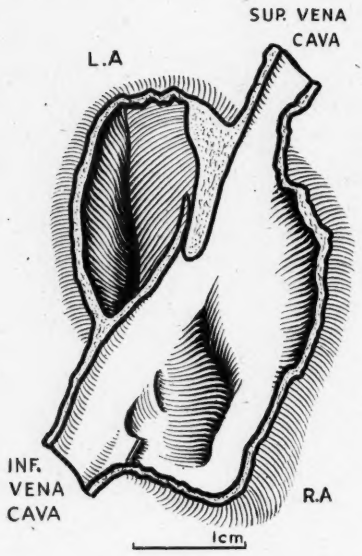

Fig. 3. Dissection of the foramen ovale in the heart of an infant aged 3 days. Note the septum primum applied against the septum secundum.

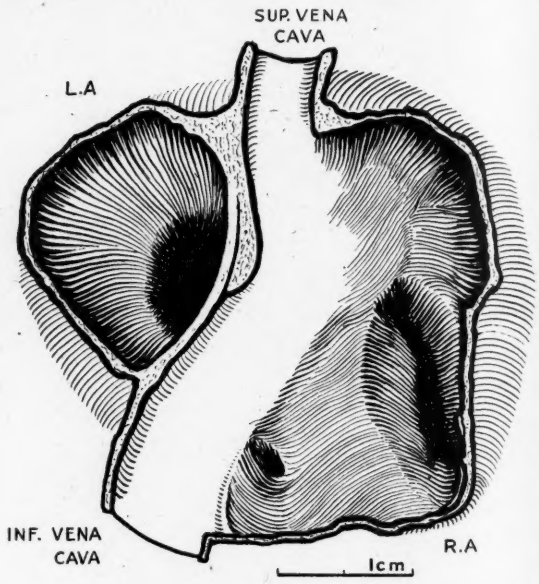

Fig. 4. Dissection of the foramen ovale region in the heart of an infant. Note the septum primum structurally fused with the septum secundum.

equal share in forming the apex of the heart and were alike in respect of the thickness of their muscle walls in a cross section. The planimetric ratio of the left to the right ventricular walls is therefore expressed by the numeral 1.

In order to meet the objection that the ratio of the wall musculature may not be representative near the apex, all the hearts were also cut across halfway between the atrioventricular groove and the apex, along a line parallel to the coronary sulcus, and similar measurements were made at that level. In the adjoining table both figures are given, the mid-level ratio in the first column, that nearer the apex in the second column. The difference is usually more accentuated near the apex, but there are exceptions. The mean of the two results seen in column 3 probably expresses the relation between the ventricles more accurately than either of the previous results alone.

Within a very short time of birth the differential growth changes in the ventricles begin. The youngest heart in my series showing such changes was that of an infant aged 6 days.

Table of planimetric ratios between the left and right ventricular walls

Age	Ratio L/R: Midway between atrio-ventricular groove and apex	Midway between preceding level and apex	Mean ratio
Before birth or less than 3 days old, mostly still-born (12 hearts examined)	1·0	1·0	1·0
6 days	1·1	1·6	1·3
3 weeks	1·4	1·6	1·5
4 weeks	1·6	2·9	2·2
7 weeks	3·0	3·0	3·0
2 months	1·9	2·1	2·0
3 months	1·5	2·4	1·9
5 months	3·9	3·1	3·5
5 months	5·5	5·1	5·3
6 months	4·4	5·2	4·8
12 months	5·6	8·8	7·2
3 years	3·4	2·7	3·1
Adult	2·4	3·2	2·8
Adult	2·9	4·0	3·4

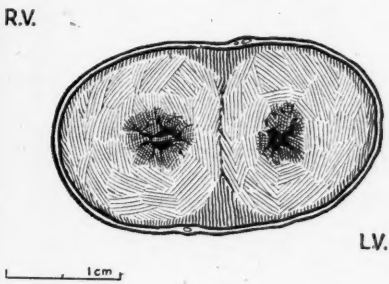

Fig. 5. Section across the ventricles of the heart of a full-term foetus.

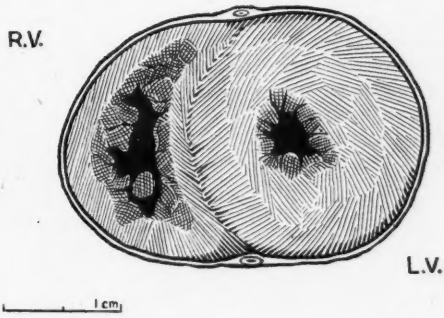

Fig. 6. Section across the ventricles of the heart of an infant, aged 3 months.

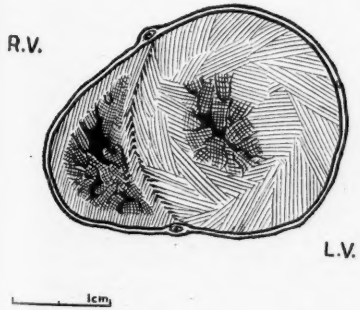

Fig. 7. Section across the ventricles of the heart of an infant, aged 5 months.

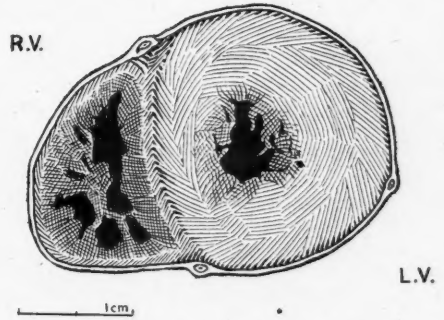

Fig. 8. Section across the ventricles of the heart of an infant, aged 12 months.

The right ventricle wall was already thinner than that of the left ventricle. With increase of age up to 12 months there was a progressive increase in the ratio of left to right ventricle (see table). The progression in the ratio according to age does not appear to be regular, but this is no doubt due to the fact that data are only available for single hearts of different ages, with all the possibilities of individual variation. The heart of an infant aged 12 months gave the high ratio of 8·8 near the apex (Fig. 8), with a mean ratio of 7·2. The wall of the right ventricle in this heart was extremely thin as compared with the left side. The calculations in adults were made on two apparently normal hearts obtained at random in the dissecting room. No heart in the series showed any congenital defect.

The numbers expressing the ratios between the left and right sides are suggestive, but the observations are of course not sufficiently numerous to allow a final statement on the rate of the growth change. It appears from this investigation that the adult proportions between the left and right ventricles are already present about the age of 3–5 months (Figs. 6, 7); the figures for the short series presented here, however, suggest the possibility that the right ventricle continues to become thinner, until the high ratio of 8·8 near the apex may be seen at the age of 12 months (Fig. 8). If this is so, at some period after the age of 12 months a growth change in the opposite direction must take place, until the adult proportions are reached once more. The heart of an infant, aged 3, gave the mean ratio of 3·1.

DISCUSSION

On purely anatomical grounds Sabatier (1791) evolved the conception of two caval streams in the right atrium. He described the inferior caval stream as being shunted by the Eustachian valve through the foramen ovale into the left atrium, and similarly he postulated the superior caval stream to be directed to the right atrio-ventricular orifice by the intervenous tubercle. The theory gained general favour and remained the accepted view of anatomists and physiologists for more than a century.

The theory of the unmixed caval streams was attacked by Pohlman (1909), and later by Kellogg (1928, 1930), who based their criticisms on the results of very ingenious experiments with living foetal pigs and dogs. Kellogg claims that 'the Sabatier conception is a mere philosophical argument, based on the supposed advantages that would accrue from a differential segregation of pure and impure blood'. More recently Windle & Becker (1940) have shown by experiments on living foetal cats and guinea-pigs that there is undoubted differentiation of the two currents in the right atrium, provided the heart is beating vigorously. They contend that the Pohlman-Kellogg conclusions rested on false premises. For various reasons connected with the technique of the experiments, the foetal animals in the Pohlman-Kellogg series were not in a normal state and the hearts were beating feebly. In such feebly beating foetal hearts Windle & Becker showed that the two venous currents mix freely in the right atrium.

The inspection of the foramen ovale and of its associated valves in the human foetal heart leaves no doubt that the main volume of the inferior vena cava stream runs through the foramen ovale to the left side of the heart, and that the superior vena cava current is directed towards the tricuspid orifice by the bulge of the septum secundum, the 'intervenous tubercle' of some writers. A Eustachian valve, in the form of a raised fold of endocardium lying between the inferior caval opening and that of the coronary sinus, is seen in most specimens, but it is variable. The Eustachian valve is well marked in Fig. 1, and still quite obvious in the infant's heart shown in Fig. 4 although the foramen ovale is closed. In the hearts shown in Figs. 2 and 3 the valve is not so obvious, but a fold of endocardium can always be detected in that region. When the Eustachian valve is well developed its line runs towards the bulge of the septum secundum (Figs. 1, 3). The anatomical appearances in the human foetal heart confirm the authoritative statement made by Franklin (1940) that the foramen ovale lies at the termination of the inferior vena cava[1] and that both structures are 'outside the right atrium proper'.

The close resemblance of the two ventricles in the foetal heart and the equal thickness of their muscle walls (Fig. 5) are readily explained on physiological grounds, and support Pohlman's studies on the ventricular capacities which constitute the first set of experiments

[1] In a more recent publication (Amoroso, Franklin & Prichard, 1941) the term posterior (inferior) caval channel is used to define this physiological channel, in contradistinction to the term inferior vena cava which refers to an anatomical entity.

in his article (1909). Most observers are agreed that the capacities of the two ventricles in the foetal mammal are the same and that the right and left ventricles maintain equal pressures and expel equal quantities of blood. Pohlman proved this point for the foetal pig's heart.

More blood enters the right atrium by the umbilical vein and inferior vena cava than by the superior vena cava. Therefore some of the inferior caval stream must be turned over to the right side if the two ventricles are to receive the same amount of blood. The modern view has been summarized by Franklin (1940) as follows: 'The whole of the superior caval flow passes into the right atrium and thence into the right ventricle. The bulk of the inferior caval flow goes from the inferior vena cava via the foramen ovale into the left atrium, and thence into the left ventricle. A small portion of it, however, passes into the right atrium and right ventricle.' The anatomical conditions met with in the present series of hearts are in harmony with this conception.

The older anatomists probably laid too much stress on the qualitative differentiation of the two blood streams, while the emphasis should be on the quantitative side. Nevertheless, the qualitative differentiation is important. Recent experimental work, supported by these observations in human hearts, goes to show that a definite separation between the relatively pure blood entering by the inferior vena cava and the impure blood entering by the superior vena cava takes place in the right atrium.

SUMMARY

Dissections to show the foramen ovale in the hearts of full-term foetuses and newborn infants are described. The anatomical evidence strongly supports the view that the two caval currents remain unmixed.

Attention is drawn to certain differential growth changes which take place in the ventricles as a result of the changed circulation after birth.

I gratefully acknowledge a grant from the University of Cape Town towards the cost of this investigation.

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THE SQUATTING INDEX OF THE FEMORA OF PUNJABIS

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Martin (1932) noticed the increased depth of the intercondylar fossa in the femora of squatting as compared with non-squatting races. The increased depth of the intercondylar fossa in the former is attributable to the pressure on it of the posterior cruciate ligament during the extreme flexion which accompanies the squatting posture. The ratio of this measurement to the greatest depth of the articular surface of the lower end of the femur can be expressed as an index, which varies in different races. This investigation was taken up by Siddiqui (1934, 1936) who examined 198 femora from Indians of the United Provinces, and 100 femora from modern Canadians.

In the present work 200 femora from Punjabis have been examined. The lowest social strata of Punjabis adopt the squatting posture both for rest and work. The bones used for the investigation were obtained from the Dissection Hall of the King Edward Medical College, Lahore; they had been previously identified and marked as belonging to genuine residents of the Punjab. The study was carried out on lines similar to those of Martin and Siddiqui. For details of the technique adopted, reference may be made to their papers. The results obtained are given in Table 1 and are compared with other published results.

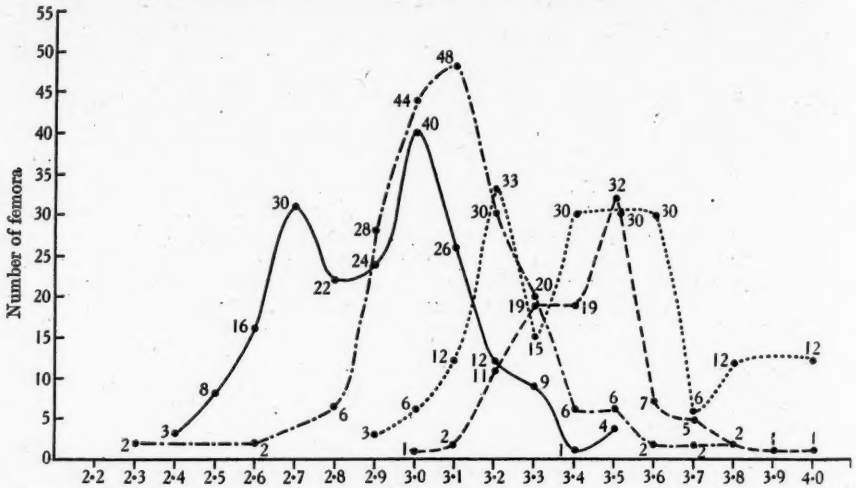

Fig. 1. — Punjabis. -·- United Provinces. – – Modern Canadian. ... Modern Irish.

Table 1

Subjects and total no. examined	No. of femora under different ratios																	
	2·3	2·4	2·5	2·6	2·7	2·8	2·9	3·0	3·1	3·2	3·3	3·4	3·5	3·6	3·7	3·8	3·9	4·0
Punjabis (198)	2	3	8	16	31	22	24	40	26	12	9	1	4	—	—	—	—	—
United Provinces (198)	2	—	—	2	—	6	28	44	48	30	20	6	6	2	2	2	—	—
Modern Irish (189)	—	—	—	—	—	—	3	6	12	33	15	30	30	30	6	12	—	12
Modern Canadian (100)	—	—	—	—	—	—	—	1	2	11	19	19	32	7	5	2	1	1

The comparative curves of the squatting and non-squatting races are given in Fig. 1. The average mean of the ratio: $\frac{\text{Greatest depth of the lower articular surface}}{\text{Depth of the intercondylar fossa}}$ for Punjabis is 2·9.

For the residents of the United Provinces it is 3·1, for modern Canadians 3·42, and for modern Irish 3·43.

To bring these measurements in line with other osteometric data, the adoption of a squatting index is suggested. For this purpose the following formula may be used:

$$\frac{\text{Depth of the intercondylar fossa}}{\text{Greatest depth of the lower articular surface}} \times 100.$$

The average mean of the squatting indices in femora of Punjabis is 34. Table 2 gives the results expressed in terms of this index.

Table 2

Total no. examined	No. of femora under different squatting indices														
	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42
Punjabis (198)	4	2	9	10	32	36	20	18	27	15	13	6	2	2	2

SUMMARY

Observations on the lower ends of 200 femora from Punjabis have been made, confirming the conclusions of other authors that in squatters the intercondylar fossa is deeper than in non-squatters. The adoption of a squatting index is also suggested.

I wish to express my grateful thanks to Professor M. A. H. Siddiqui for suggesting the subject of inquiry, and for much help and guidance during this investigation.

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Anatomical Society of Great Britain and Ireland

PROCEEDINGS

April 1942

RECORDED BY
A. J. E. CAVE

PROCEEDINGS. ANATOMICAL SOCIETY

APRIL 1942

The Annual General Meeting of the Society for the Session 1941–2 was held in the Department of Zoology, University of Sheffield, at 9.30 a.m. on Friday and Saturday 17 and 18 April 1942, the President (Dr W. L. H. Duckworth) in the Chair.

Messrs D. M. Blair, F. Davies and E. T. B. Francis read a paper on 'The conducting system of the marsupial heart', of which the following is an abstract:

In general, the disposition and neural accompaniment of the conducting system of marsupials resemble those of the placental mammal, particularly the ungulate. The S.-A. node is horseshoe-shaped, embracing the end of the right anterior vena cava; nerve cells are found in the substance of the node, as in the monotreme. No Purkinje fibres are present in the atria and there is no specialized direct connexion between S.-A. and A.-V. nodes. Differences are noted between the histological structure of the foetal and adult nodes. The cytological characters of the Purkinje fibres have a closer resemblance to those of the monotreme than to those of the placental mammal. The ventricular subendocardial Purkinje fibres do not penetrate the thickness of the walls of the ventricles. Minor differences between the disposition of the left limb of the A.-V. bundle of *Macropus robustus* on the one hand and those of *M. giganteus* and *M. ruficollis* on the other are attributed to specific variation.

Professor J. P. Hill commented upon the parallel evolutionary development of the avian and mammalian cardiac conducting systems, matched by those similarities of primitive streak formation in these two vertebrate classes induced by the requirements of active mesoderm production. The approximation of the marsupial to the monotreme type of conducting system harmonizes with certain observed ontogenetic affinities between these groups. Professor Blair and Dr Francis discussed the incidence and nature of the 'ballooning' of muscle fibres encountered in the wallaroo heart, a curious and abrupt alteration in fibre calibre, very common in both the atrial and the ventricular musculature.

Professor F. Davies and Dr E. T. B. Francis read a paper on 'A graphical method of comparing the glycogen content of muscle fibres from various regions of the heart', of which the following is an abstract:

The method consists of projecting on to squared millimetre paper, at a high magnification, 5 μ serial sections of the frog's heart stained by alkaline carmine (controlled by the saliva test). The outlines of groups of muscle fibres are traced and the areas of the red-stained glycogen blocked out. The reliability of the method is discussed. The method indicates more accurately the *relative* glycogen content of the *muscle fibres* from various parts of the heart than does the chemical method of estimation, in which no allowance is possible for the relative amounts of 'inert' fibrous tissue in the masses analysed, and which thus gives results different from those obtained by the graphical method, particularly in those regions where the fibrous tissue is abundant. The method stresses the importance of histological control of purely chemical estimations of this kind.

The President commented appreciatively on this paper, as exemplifying the value of chemico-biological collaboration. Professor Harris inquired as to the method adopted of fixation of the fresh material used, with particular reference to the prevention of glycolysis therein. Professor Davies, Dr Francis and Dr Winter gave details of the chemical and histological techniques employed: glycolysis being rapid in the mammalian, but slow in the amphibian heart, the precautions usually taken when fixing mammalian tissues were applied to the amphibian (salamander, frog) cardiac muscle used. Uncontrolled chemical estimations of the glycogen content of heart chambers were unreliable and even misleading.

Professor F. Davies and Dr E. T. B. Francis read a paper on a 'Cinephotographic method of analysis of the cardiac rhythm'.

The method consists of projecting successive frames of a slow-motion cine-film of the normally beating salamander heart on to paper and of tracing thereon the magnified outlines

of the various cardiac chambers. By the superimposition of such tracings, the detailed course of a single contraction wave is followed throughout the whole heart, whilst from them is constructed a graphical analysis of the various phases of a complete cardiac cycle. The authors compared this method with established electrical methods of cardiac cycle analysis: the changes revealed by the graphical method are surface changes only and are uninformative as to changes affecting the deeper parts of the heart. The course taken by the cardiac impulse, as revealed by cinephotographic analysis, was that already postulated on purely histological grounds.

The PRESIDENT commented on the accuracy and precision of the authors' findings.

Mr W. E. ADAMS read a 'Note on the autonomic system of *Lacerta*'.

The PRESIDENT suggested that the course of the deep cervical nerve relative to the cervical rib elements would prove helpful in determining the nerve's homologies: Gadow had observed this nerve to pass dorsal to the cervical pleurapophyses in the crocodile. Professor BOYD commented upon the difficulties inherent in the study of the reptilian sympathetic system: he considered that the supposed preganglionic fibres mentioned might more likely be viscero-sensory in nature, and that the sympathetic played but a minor part in the innervation of the carotid body region: he inquired as to the relation of the deep cervical sympathetic trunk to the vertebral artery. The author replied that the possibility of the visceral afferent nature of the so-called preganglionic fibres was being borne in mind during the present research, as yet incomplete, that there was no vertebral artery in *Lacerta viridis*, but that the deep cervical nerve was the homologue of the human nervus vertebralis. Mr BELLAIRS, inquiring about the innervation of the lateral orbital gland, was answered that the present investigation had not included particular attention to this point: an innervation from the infraorbital nerve had alone been casually observed. Professor DAVIES suggested an investigation of cell groups in the spinal cord as likely to throw light upon the question of preganglionic fibres in certain spinal nerves, and Dr BACSICH spoke upon the need for establishing clearly the morphology and homologies of the deep cervical nerve. Mr ADAMS replied.

Professor J. D. BOYD read a paper upon 'Carotid-body-like cells in the adventitia of the ductus arteriosus of the pig'.

Cells identical in appearance with those of the carotid body are observable in the ductus arteriosus adventitia of various pig embryos and of the 10-day old pig. Such cells are richly innervated and are intimately related to autonomic nerve cells. The author demonstrated and discussed their relation to comparable cell masses in other mammalian species.

Professor APPLETON referred to the migration of vagal ganglion cells into the thoracic cavity and asked whether the cell masses here described did not represent a similar migration of carotid-body-like structures. The author replied affirmatively, but stressed that the real problem concerned the ultimate nature of these migrating cells. Professor DAVIES was answered that these ductus arteriosus cell groups were not composed of chromaffin cells. Professor WOOD JONES pleaded for a comprehensive, yet precise and convenient, nomenclature for the system of scattered, peculiar cell masses described by the author in this and in previous communications.

Professor W. E. LE GROS CLARK read a paper on 'The Meynert cells of the visual cortex'.

The PRESIDENT asked whether the horizontal processes of the Meynert cells contributed at all to an inner stria and was answered affirmatively. Professor DAVIES, inquiring about experimental changes, was informed that chromatolysis was confined to the operated side of the cerebral cortex, following section of the cortico-tectal tract. Professor BOYD briefly discussed the relation of the mesencephalic lesion to the pretectal nucleus.

Dr G. WEDDELL read a paper on 'The anatomical basis of regenerative hyperaesthesia', and, with Dr GLEES, a paper on 'The regeneration of cutaneous nerve fibres'. These papers were discussed jointly.

Professor WOOD JONES criticized the authors' usage of the term 'causalgia', which was nowadays employed in a sense wholly different from its original. He inquired concerning the reputed re-establishment of cutaneous sensation on the basis of ingrowths from local nerves, and also concerning the anatomical basis for protopathic sensibility. Dr WEDDELL replied that the re-innervation of cutaneous areas from neighbouring nerves had been demonstrated

by experimental nerve block. Each cutaneous sensory spot is normally innervated by multiple fibres. Head's conception of the anatomical basis of his 'protopathic' sensation was not substantiated, for during regeneration there was found to be a numerical reduction of nerve endings of all types per unit area of skin. Certain types of causalgia, e.g. thalamic pain, have no peripheral basis.

Professor HARRIS discussed Head's 'neuralgia of regeneration'. Dr FRANCIS observed that the term 'nerve net' was employed, in invertebrate anatomy, in a sense quite different from the authors. Professor LE GROS CLARK commented upon the curious nerve endings observable in painful scars and inquired how precisely they gave rise to painful sensations. Mr ADAMS inquired concerning the role of the Schwann cells in the regenerating nerve fibre. Dr WEDDELL, in reply, said he could offer no explanation of the actual mechanism of pain production in scars.

Professor T. NICOL read a paper on 'Further observations on normal fat distribution in the reproductive tract of the female guinea-pig during the oestrous cycle and its possible use as a control in ovarian hormonal experimental work', of which the following is an abstract:

Examination by Sudan III staining of the reproductive tract of forty-five guinea-pigs confirmed a previous observation that there is normally a cyclic distribution of fat in the uterine horn epithelium: it revealed also a cyclic fat distribution in the uterine body, cervix, vagina, and vaginal smear. The maximum amount of fat in the uterine horns and body occurs during the luteal phase of the cycle, whilst the fat appearances in the lower cervix and vagina occur at oestrus only. The fat is non-luminous in polarized light and is probably neutral fat.

The fat in the uterine horns is probably related to the process of preparation of the uterus for implantation and nourishment of the ovum, whilst the fat in the distal uterine body may be associated with the growth changes occurring there. There is no evidence to suggest that these appearances indicate degeneration: on the other hand, those in the cervix and vagina represent fatty degeneration.

Experimental work confirms the belief that the fat in the uterine horns and body is influenced by the luteal hormone, whilst the fat appearances in the cervix, vagina and smear are controlled by the follicular hormone.

It is suggested that the normal maximum occurrence of fat in the uterine horns might be used as a control for experimental work with the luteal hormone. From preliminary experiments it appears that the amount of fat produced is related to the amount of luteal hormone injected, and may depend also on the preparation used and the mode of sensitization of the uterus by follicular hormone.

Professor HAMILTON discussed the histological changes occurring in the uterine tract of the polyoestrous cavy and other mammals, remarking that degenerative changes such as those described are but to be expected. He asked how a distinction could be drawn between the fat of degeneration and the fat provided for embryonal nutrition after implantation. Dr WYBURN asked for a precise definition of 'luteal' phase, and from personal experience of experimental work held that the cavy oestrous cycle was not strictly comparable with that of the monkey and other laboratory animal types. Professor HARRIS discussed protein degeneration in relation to the appearance of fat, and fat storage as a source of readily available energy. In reply the author admitted the great difficulty of distinguishing between nutritional and degenerative fat, particularly since the fat cells reveal no actual histological breakdown: there seemed no reason for degeneration in the uterine horn in the middle of the cycle. A luteal phase was not well marked in the cavy.

Professor T. NICOL read a paper on 'Fat distribution in the uterine horns of the ferret during the follicular phase of the oestrous cycle and during pseudo-pregnancy', of which the following is an abstract:

Ferret uteri were stained for fat by Sudan III and osmic acid during the follicular phase of the oestrous cycle and during pseudo-pregnancy.

Fat was found to be absent from the uterine horn epithelium during the follicular phase of the cycle and during the earlier stages of pseudo-pregnancy, when intensive growth is occurring in the surface and glandular epithelium. Fat first appears in the uterine epithelium about the 31st day of pseudo-pregnancy: from the 38th to the 43rd day the surface epithelium shows intense fat staining, the fat-containing cells being two or three layers deep, and desquamating

into the uterine lumen. At the 47th day the surface epithelium shows a single layer of flattened cells, intensely fat-stained; many fat-containing cells lie free in the uterine lumen, the glands are lined now with cubical epithelium and the connective tissue cells are increased in number. No fat is present at the 53rd day, when the uterus appears to have reached a resting stage. The change from the swollen epithelial cells of pseudo-pregnancy to the cubical epithelium of the resting uterus appears to be relatively abrupt. The fat is non-luminous when examined in polarized light and is probably neutral fat.

In contrast to findings in the guinea-pig, the fat appearances in the uterine horn of the ferret during the luteal phase of the cycle appear to be those of degeneration, and suggestive of the occurrence of fatty degeneration as part of the process whereby the pseudo-pregnant uterus returns to normal.

Professor HAMILTON, commenting on this communication, said that the ferret provided a clear-cut cyclical histology and an equally clear picture of degeneration; hence there appeared no reason why a correlation should not be attempted between pseudo-pregnancy in cavy and ferret.

Drs P. BACSICH and G. M. WYBURN read a paper on 'The response of the immature female guinea-pig to gonadotropic (Antostab) stimulation', of which the following is an abstract:

Previous experiments assessed the results of direct oestrogenic stimulation of the immature female guinea pig (by injection of oestradiol monobenzoate). The present work concerns the oestrous changes of the immature female guinea pig elicited by indirect oestrogenic treatment in the form of injection of the gonadotropic substance Antostab (Boots, Ltd.).

Sixteen immature, but not hypophysectomized, guinea pigs were utilized. Antostab was injected every alternate day until the vaginae opened, whereupon treatment ceased. The animals were killed when cornification in the vaginal smears became evident. The ovaries were greatly increased in weight, contained large follicles and showed changes to be attributed mainly to the follicle-stimulating factor. Changes in the uteri were on the same reduced scale as those observed after direct oestrogenic stimulation, such findings confirming the previously established inability of the immature uterus to respond maturely to hormonal stimulation.

In reply to a question by Professor HAMILTON, the authors stated that no particular part of the uterus seemed constantly non-reactive: different parts of the organ exhibited the reaction in haphazard fashion and the vaginal reaction likewise followed no definite stage-sequence.

Drs P. BACSICH and G. M. WYBURN read a paper on 'Stilboestrol treatment of the spayed female guinea-pig', of which the following is an abstract:

Experiments were undertaken to obtain data for the comparison of results of stilboestrol treatment of ovariectomized guinea-pigs with results previously (1941) obtained by administration of oestradiol monobenzoate, with particular reference to experimental vascular response. Twenty-five animals were utilized, groups of these being given (after preliminary priming) increasing doses of stilboestrol until the final group of five received a daily injection of 1 mg. on the 5 days preceding death.

A daily priming dose of 0·0025 mg. stilboestrol for 6 days was necessary to obtain vaginal opening in 100 % cases, as compared with 0·0005 mg. oestradiol monobenzoate: the vaginal smears taken showed, however, a more precocious and intense cornification. The uteri of all the animals killed were stained by the benzidine method; they were found to be enlarged and hyperaemic and to show endometrial proliferation, etc. The typical uterine antimesometric hyperaemia was present only in the last group of animals (three out of five), each of which received, after vaginal opening was established, a total of 5 mg. stilboestrol.

In a previous experiment 70 % of animals gave (after priming) the characteristic vascular response after receiving two injections of 0·5 mg. oestradiol monobenzoate (a total of 1 mg.). Thus, in terms of the establishment of vaginal opening and complete uterine response (including antimesometric hyperaemia), the ratio of biological potency of stilboestrol and oestradiol monobenzoate is as one to five.

This communication was briefly discussed by Professors NICOL and HAMILTON, to whom the authors replied.

Professor H. A. HARRIS read a paper on 'Principles of anatomy', of which the following is an abstract:

Principles of anatomy are of two kinds:

(1) Utilitarian, based on the need for that treatment of the patient which is precise, purposive and final, with special regard to health and earning capacity.

(2) Scientific or transcendental, based on a succession of hypotheses, laws and experiments which, though of no immediate clinical value, may acquire clinical value later and suggest social implications of great significance.

The two kinds of principles are discussed with reference to the anatomy of the various systems of the human body, with a view to showing that the transcendental of to-day may be the utilitarian of tomorrow. The so-called minutiae of anatomy may be the only safe landmarks in the recognition of the individual and the species, e.g. finger prints and structural features of the hair.

A discussion followed.

Mr J. J. PRITCHARD read a paper on 'Some experiments on the regeneration of membrane bones', of which the following is an abstract:

Little has been recorded concerning the mode and extent of regeneration of the cranial membrane bones. To trace the stages of regenerative repair, portions of the parietal and of neighbouring bones were removed in rats and rabbits and the operative field studied microscopically at varying subsequent intervals. Animals ranging in age from 2 days to 18 months were used. The osteoblastic cambium layer came away with the excised cranial fragment.

Operative sequelae differed markedly in neonatal and in more mature animals. With intact dura, complete parietal excision in a 2-day rat resulted in a marked cellular proliferation of the dura, and osteoblasts surrounding small islands of newly formed bone were observable. After the 6th week these islands had proliferated and had begun mutual fusion; the re-formed sagittal suture appeared normal in position and characters; macroscopically the re-formed parietal was normal though the subjacent dura remained thickened.

Concomitant removal of dura and cranial fragment was not followed by bone regeneration, and the resultant gap was closed by fibrous tissue only.

At and after the 6 weeks old stage bone regeneration never occurred, irrespective of the fate of the dura. Slight marginal growth only of new bone took place, the gap being largely closed by thick fibrous tissue. (Comparable phenomena were observed in the microscopic examination of a surgical trephined human skull.) A similar initial osteoblastic reaction was obtained in adult rats following division of the parietal to simulate fracture.

These, and relevant experiments, suggest that the dural cells of the neonatal animal are capable of producing osteoblasts de novo, a capacity lost in older animals wherein the intense fibrous reaction of the mature dura may further hinder successful regeneration.

Professor APPLETON spoke appreciatively of the author's experimental work. Professor LE GROS CLARK asked whether, after bilateral parietalectomy and unilateral prevention of regeneration, the regenerating contralateral parietal crossed its former boundaries: the author replied in the negative. Professor WOOD JONES discussed the topographical restriction of growing and regenerating membrane bones by suture lines, with particular reference to the multiple sutural ossicles encountered in markedly hydrocephalic crania. Professor BLAIR and Mr WALLS referred to Macewen's pioneer attempts to close surgically produced cranial gaps by the replacement of osseous fragments: Macewen's successful claims had not been upheld by later practitioners of his method. Professor CAVE remarked that in animals, at least, fractures of the facial membrane bones were characterized by permanent suture formation, and that such sutures had been misinterpreted as indicating adventitious facial elements. Dr HAINES remarked that callus formation at some distance from a wound, said to be determined by purely mechanical factors, was more likely the result of hormonal activity. Professor HARRIS commented on the low osteogenic power of membrane bones, particularly as indicated by the close proximity thereto of various nerves, by the absolute or practical absence of callus formation following cranial fractures in the newborn, and by the great time taken by sequestrum separation.

Mr H. Hughes read a paper on 'The evolution of the human transverse carpal arch'.

The President inquired concerning the nature of the ossicle depicted at the apex of the developing ulna: he suggested that the development of pronation and supination in eutheria was worthy of investigation as being intimately correlated with the structure and function of the wrist-joint. Professor Appleton commented appreciatively on the paper as demonstrating the value of comparative studies in elucidating anatomico-physiological relationships. Dr Haines said the assumption that many of the larger joints of man were phylogenetically derived from a confluence of multiple discrete articulations was entirely erroneous and the paper provided valuable morphological evidence to the contrary.

Professor A. B. Appleton read a paper on 'Postural variation of the vertebral column and lower limb joints in the standing attitude', of which the following is an abstract:

Measurements of posture have been made on 119 males and 68 females (young adults) and the interrelationships of pelvic tilt, pelvic carriage and spinal form have been ascertained. Forward carriage, i.e. a forward position of the pelvis relative to the feet, is mainly attributable to ankle dorsiflexion; backward carriage depends on knee flexion, not to any appreciable extent on ankle posture. Pelvic carriage is extremely variable; the hindermost part of the sacrum may be almost 4 in. anterior, or 2 in. posterior, to the vertical plane through the heels. Pelvic carriage is important in determining the relation of the line of gravity to the feet.

The degree of static compensation for differences of carriage afforded by spinal posture has been ascertained by determining individual differences in the line of gravity and the form and orientation of the spine. Subjects with forward pelvic carriage show, generally, no spinal postural compensation; those with backward carriage manifest some such compensation, effected by a lumbar and lower thoracic flexion.

Variations of pelvic tilt are independent of knee- or ankle-posture, but are dependent on hip-joint posture. In the standing attitude the hip joint has never been found to be fully extended. Differences of pelvic tilt affect but little the orientation of the upper spine and tend to be associated with differences of form of the lumbar spine.

The various forms of spine have been analysed: both pelvic tilt and pelvic carriage are found to influence substantially spinal form in the standing attitude. The posture of the pregnant female is distinctive, with a backward carriage due to knee-flexion, a diminution of pelvic tilt and a lumbar lordosis.

The President commented on the value of this paper from the standpoints of anthropometry and physical culture. Professor Harris discussed the author's terminology, pleading for a more precise definition of posture, position and stance, in view of prevailing differences and discrepancies of interpretation, especially among masseurs, orthopaedicians and clinicians.

Dr R. Wheeler Haines read a paper on 'Eudiarthrodial joints in fishes'.

Professor J. Kirk read a paper on 'The histology of the pancreatico-biliary ducts, with particular reference to the so-called ampulla of Vater and to the sphincter of Oddi', of which the following is an abstract:

The histology of the extra biliary ducts has been studied in foetal and adult human material.

In five adult specimens examined no Vaterian ampulla was found. The intraduodenal portion of the common bile duct appears as a narrowing channel, 'packed' with villous processes in a valve-like manner well adapted for prevention of regurgitation. No sphincteric arrangement of muscle fibres is observable distal to the site of perforation of the gut wall by the common duct. At the site of perforation there occurs a distinct thickening of the bowel's circular muscle coat: here the common duct is surrounded by circular muscle fibres and Auerbach's plexus appears to be considerably developed. As the duct proceeds through the submucosa, fibres from the circular muscle coat of the duodenum are continued into its wall and may be traced distally as longitudinal fibres in the connective tissue core of the villous processes.

In no specimen has any sphincter been found at the duct ostium. Diverticula and mucous glands are abundant in the duct walls and increase towards the ostium, extending beyond it and conferring upon the duodenal mucosa its typical appearance of mucous crypts discharging upon an avillous surface.

Histological examination of the biliary passages for muscle fibres has been made at varying levels proximal to the bowel: at no point has any sphincteric disposition of fibres been encountered. Paucity of muscle is characteristic of the duct walls. No trace of a sphincter occurs at the junction of common hepatic and cystic ducts. The common bile duct may have a substantial companion nerve.

Certain of these present findings are correlated with clinical problems.

Professor CAVE exhibited drawings of the conspicuous extraduodenal, or intramural, pancreatico-biliary ampullae of various mammals, to show by contrast the retrogressive condition of the relatively rare human ampulla, and to demonstrate the essentially intestinal nature of the ampullary musculature.

Mr A. d'A. BELLAIRS read a paper on 'Jacobson's organ and the orbital glands in certain snakes and lizards', of which the following is an abstract:

Recent experimental work (Noble, G. K. 1937, *Bull. Amer. Nat. Hist. Mus.* **73**, 673) has indicated the important role played by Jacobson's organ in the behaviour of many snakes and lizards. It is now generally believed that the nature of odorous particles is appreciated by their collection on the tips of the bifurcate tongue and their deposition at the orifices of the ducts of Jacobson's organ which open in the roof of the mouth on each side of the midline.

The structure and relations of the organ have been previously described in *Vipera berus* (Bellairs, 1942, *J. Anat., Lond.*, **76**), and observations have been made subsequently in the burrowing snake (*Typhlops diardi*) and in other species. In *Typhlops* Jacobson's organ is extremely large, occupying much of the front region of the tiny head; its duct into the mouth is relatively much wider than in *Vipera*, and the disposition of its bony capsule and of the nasal cavity differs considerably from the corresponding conditions in *Vipera*.

The great orbital (Harderian) gland has been identified in all representatives of the principal families of snakes and lizards. In lizards a true lachrymal gland, situated lateral to the eyeball and lubricating the nictitating membrane, is also present, except in those species wherein, as in snakes, the eyelids have been replaced by a spectacle. In lizards the great orbital gland, lying in front of the eyeball, discharges into the conjunctival sac (when present) and also into the duct of Jacobson's organ. In snakes, wherein both nictitating membrane and conjunctiva have been lost, the gland, generally situated behind and medial to the eyeball, supplies Jacobson's organ only, and no intrinsic orbital glands are identifiable.

Professor WOOD JONES commented on this paper, and referred to a correlation between the development of Jacobson's organ and the habit of feeding in the dark. In birds the Harderian (supraorbital) glands opened into the nasal chambers, a point well seen in the Tubinares, the only birds possessing an olfactory sense: the glands were notably large in shore-feeding birds. Dr FRANCIS regarded the paper as replete with fruitful suggestions for further enquiry and recommended a detailed comparative study from the Urodeles upwards. The PRESIDENT praised the author's prosectorial skill and presentation.

REVIEW

Cytology and Cell Physiology. Edited by G. BOURNE. (Oxford University Press.) 1942. Price 20*s*.

This is an admirable collection of reviews of a number of aspects of cytology by contributors who have themselves made notable investigations in the fields they consider. It is well printed, has excellent illustrations and can be said to be a distinct credit to contributors, editor and publisher. It does not pretend to cover the whole field of cytology, and the anatomist may be disappointed at the emphasis on the chemical and physicochemical aspects of the problems considered, at the expense, he may think, of morphological questions. But there is plenty of morphological cytology in the book, and the manner in which micro-chemistry and modern physical techniques are shown to have solved and to be solving problems long considered to be purely morphological is surely a sufficient justification for the bias.

The subjects covered range from cytological technique through structure of cytoplasm, cell surface, cytoplasmic organelles, nucleus and chromosomes to the enzyme systems of cells. It is invidious to select certain of the contributions for special attention as there is a pleasing uniformity in level and in interest. Having made this statement, however, it is perhaps excusable to draw attention to the contributions which are likely to be of rather more interest to the anatomist and histologist. Dr J. R. Baker, in the first chapter, deals with some aspects of cytological technique. This is a valuable survey with useful advice on technical details and a most salutary emphasis on the necessity for studying fresh, as well as fixed, material and on the need for getting away from purely empirical staining to a rational histology based on histo-chemistry. He also stresses the need for a proper appreciation of the possible achievements, both positive and negative, of the microscope. He emphasizes the necessity for studying *substance* as well as *structure*, an emphasis which may be necessary at this stage of our knowledge when histology is so largely an account of the structure of highly modified, and often much distorted, dead cytoplasm and when we know so little about the chemical and physicochemical constitution of cytoplasm. But the reviewer believes the antithesis to be a false one—structure, on the biological level ultimately resolves itself into a problem of inter- and intra-molecular arrangement, and when we have reduced the cytoplasm to a description in terms of molecules surely we have reached *substance* in Dr Baker's sense of the term. Doubtless, then, the attempt will be made to reduce the description to the terms of electron chemistry and physics and *substance* will automatically become whatever physical science of the time will hold as its considered opinion of the 'stuff' of the universe. But now when physicists are so vague, and philosophers so confused, on what is substance, it seems safer to avoid the term. The problem is the resolving of gross and microscopic structure into ultramicroscopic and molecular structure.

And, indeed, it is with this resolution that a great deal of the rest of the book is concerned. Dr J. F. Danielli, in two chapters, surveys modern work on the physicochemistry of the cell and cell surface in terms of ultra-structure, and in doing so summarizes modern techniques devised to study the many problems which have arisen in the course of this work. In this regard Dr Schulman has contributed a short chapter on the technique of studying monomolecular films which, however, assumes more knowledge on the part of the reader than the reviewer was able to supply. Dr Danielli's contributions are extremely interesting, but have a terseness (dictated doubtless by space considerations, for the problems he considers would provide material for many volumes) which detracts from their value. In particular the reviewer feels that a more detailed consideration of the X-ray analysis of fibre structure would have been advantageous; in fact it is surprising that a separate chapter has not been devoted to the implications and applications of the work of Astbury, Bernal and other protein crystallographers. The structure of protein molecules and the orientation of such molecules and their aggregations are the very basis of the structural analysis of cytoplasm and must represent for the anatomist, as for the cellular physiologist, the *terminus ad quem*. It is indeed extraordinary, as Picken has pointed out recently (*Biol. Rev.* 1940, **15**, 133) how far our modern knowledge has rehabilitated and extended the ideas of the botanist

Näegli on micellar structure, ideas which formed, as early as the middle of the last century, a rational basis for the study of ultimate biological structure. Like so many fundamental contributions Näegli's work was ignored and obscured by the post-Darwinian concentration of biologists on the details of the evolutionary story, especially recapitulation. But modern biologists can well say 'nous avons changé tout cela', and how far the position has been changed is demonstrated in Dr White's admirable chapter on the nucleus, chromosomes and genes. This chapter is concerned almost exclusively with modern work on the structure of the chromosomes and shows how complicated this structure is, a demonstration which enables one to agree readily with the author's statement that the original picture of the chromosome as a linear sequence of genes was merely a first approximation to the truth. The newer work, recorded by White, deals with the centromeres, telomeres and the eu- and hetero-chromatic regions of the chromosomes and it may also be an approximation, but, when considered in relation with the knowledge of nucleic acid metabolism so clearly stated by Dr White, one feels that not only has there been an advance but that the advance is in the right direction.

Other contributions to the volume are an account of mitochondria and the Golgi apparatus by the editor, a summary of the technique and of the findings of micro-incineration by Dr Horning, a description of known enzyme systems of cells (which includes the best general account of the fundamental work by Linderstrom-Lang and Holter of the Carlsberg Laboratory on the distribution of cellular enzymes that the reviewer has met) by Drs Blaschko and Jacobson, and a chapter on the pathological aspects of cytology by Dr R. J. Ludford. The subject and author indices are full and there is an extensive bibliography in which, however, titles are not given and there is no uniformity in the treatment of initials of contributor's names. But improvement in the last regard would have added but little to what is an excellent production from every point of view. It is a book which will stimulate younger workers and which cannot but be of great use to all who have to deal with histological and cytological problems.

A final comment seems relevant. Here is a book of transcendent importance to anatomy, for it is concerned with the ultimate details out of which microscopic and macroscopic structures are built. Yet the contribution of academic anatomists to its contents are so meagre that one has to read through pages of the bibliography before coming to the name of one. Admittedly the subject now requires the treatment of skilled biochemists and biophysicists, but why should anatomists have allowed the opportunity to slip without, at least, leaving some significant mark on the field? Several reasons suggest themselves—the divorce of histology from anatomy, the unfortunate linkage with surgery, the early selection of anatomists and their incarceration in the dissecting room, the concentration on dead tissues, the heavy teaching duties—but none seem sufficient. A detailed history of anatomy and anatomists in the past hundred years might help to explain why, with so much to do so little was, in fact, done in those fields where interest in, and surmise about, structure would appear to have been a prime requisite.

J. D. B.